QUANTITATIVE ORGANIC
MICROANALYSIS

QUANTITATIVE ORGANIC MICROANALYSIS

OF

FRITZ PREGL

BY

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Medical Research in Heidelberg*

THIRD ENGLISH EDITION

TRANSLATED FROM THE FOURTH REVISED
AND ENLARGED GERMAN EDITION

BY

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PREFACE TO FOURTH GERMAN EDITION

On December 13th, 1930, F. Pregl, the founder of organic microanalysis, was suddenly called away from his full life. It was not in his nature to publish his pioneer work, which G. Goldschmiedt and E. Späth considered to be the greatest advance in the department of organic elementary analysis since the time of J. v. Liebig, until every method had been carried out as simply as possible, reliably and also with very great accuracy. Two experimental lectures in Berlin and Vienna caused a great sensation in professional circles, and from that time onwards his laboratory was sought by many professional colleagues and pupils, whom F. Pregl willingly made personally conversant with his methods. In 1917, on account of the increasing general interest in his work, he resolved to publish a monograph. This was the first edition of this book, which was followed by a second and third. Because this is not the place in which to discuss the value of F. Pregl's scientific work in medical departments nor to count up the numerous very high academic honours awarded to him, reference should be made to the obituaries in chemical and medical literature in 1931.

From the original personal teaching, there developed regular courses in the Medico-Chemical Institute of Gratz University, which attracted hundreds of pupils from all parts of the world. The experiments and observations made by F. Pregl and ourselves, his colleagues, as well as the application of his methods by his pupils in other scientific institutes and industrial laboratories, led to the working out of new methods, which are quoted in the new editions.

As the publisher, Julius Springer, asked me to edit the fourth edition of the book of my highly revered teacher, the additional new work, which has recently been worked out on his foundations, must be incorporated in his monograph.

In order to distinguish this from F. Pregl's own work, every process recommended is quoted under the name of its author. In F. Pregl's opinion, only methods which have been well verified for a long time in practice can be taken into consideration. They were carefully checked by my assistant, Fräulein L. Linge, to whom I

here express my sincere thanks, and by myself. I have also undertaken the task of giving my attention to cases where one method or another requires to be completed and where in the meantime one has to reckon with uncertainty in the valuation of the results of analyses, especially in the determination of general groups of natural products.

Through the practical part, described in detail, the book should enable everyone to learn microanalysis and should place the expert in a position quickly to refer to this or that in the laboratory.

Besides numerous improvements, resulting from the practice of my professional colleagues, to which in every process very full consideration has been given, there may here be mentioned the new methods of determination given in the fourth edition :—

The alkalimetric determination of chlorine and bromine ; the titration of iodine as iodate ; the volumetric determination of sulphur in the presence of nitrogen and halogen ; the determination of barium and phosphorus and of nitrogen and mercury, respectively, in the presence of each other ; the direct determination of equivalent weights ; the determination of " active hydrogen," the titration of amino acids; the volumetric determination of alkoxyl and alkyl imino groups, and of methyl groups attached to sulphur ; a simplified determination of acetyl and benzoyl groups and the determination of methyl groups attached to carbon, as acetic acid ; the determination of isopropylidene groups attached to oxygen and carbon, as acetone ; catalytic microhydrogenation ; photoelectric measurement of the absorption spectra ; micro-molecular refraction ; determination of molecular weight by isothermal distillation ; determination of melting-points under the microscope and of boiling-points with very small amounts of material.

The enlargement of range through the additional method has therefore caused the omission of the notes on the purifying of small amounts of material, which appeared in the third edition, and has also caused the description of historical development to be shortened.

I particularly wish to thank Dr. E. Wiedemann, Chemische Fabrik, formerly Sandoz, in Basle, who was kind enough to take the trouble to read over the manuscript. I thank Dr. J. Unterzaucher, Director of the Microanalytical Laboratory of the Technical High School, Munich, for contributions from his rich practice. I express my thanks to Dr. F. Baer for drawing up the chapter " Determination of Absorption Spectra," and to Dr. E. F. Möller for the description of the " Determination of the Number of Double Bonds by Catalytic Microhydrogenation."

And, not least, my friends Dr. A. Soltys and Dr. A. Verdino, at the Medico-Chemical Institute of Gratz University, may here be thanked for the helpful way in which they have met me during my employment at the F. Pregl Institute.

I thank the Kaiser Wilhelm Institute for far-reaching support for my work.

<div align="right">HUBERT ROTH.</div>

HEIDELBERG.

TRANSLATOR'S PREFACE TO THIRD ENGLISH EDITION

THE reasons for considering a fourth German edition of this book necessary are given fully by Dr. Roth in his preface. Besides the additional methods referred to by him, modifications of the late Professor Pregl's method of determination of carbon and hydrogen, and methods for the determination of carbon in the wet way, are given. Descriptions of the Bunge microchemical and aperiodic rapid balances and of various ultramicrochemical balances have also been added. The work has been largely rearranged by Dr. Roth and the number of illustrations increased from fifty-one to seventy-two.

The size of the present English edition has similarly been increased to 267 pages, from 232 in the second English edition. Like Dr. Fyleman, the translator has endeavoured to follow the original closely. She is indebted to his translation for considerable portions of the work, which have been included in the present edition almost, or entirely, unaltered.

It may interest readers of an English edition to know that, as early as 1878, an article by H. Chapman Rose, " On a Simplification of Regnault's Method for Determining Boiling Points," was published in the *Transactions of the Chemical Society* (**33**, p. 175). The method is very similar to that of A. Schleiermacher, published in 1891, but appears to have been unknown to him, and also to Dr. Roth, who refers only to the work of the German chemist.

<div align="right">E. BERYL DAW.</div>

EALING, LONDON.

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QUANTITATIVE ORGANIC MICROANALYSIS

A. MICROCHEMICAL BALANCES

Historical

In the year 1906 W. Kuhlmann in the works of P. Bunge, who had lost his eyesight, had already constructed an " Assay Balance for Precious Metals," which, with a maximum load of 20 gm., weighs very accurately to 1 hundredth of a milligram. F. Emich mentions it in a lecture on microanalysis in 1910 and calls it, in later communications, " Kuhlmann's small analytical balance." F. Pregl's first microanalyses, which were published in Abderhalden's handbook (1912), were carried out with this balance and, as F. Pregl himself said, with a proved accuracy of " only " 1 hundredth of a milligram.

In general work and after personal interviews with F. Pregl, Kuhlmann developed from the assay balance, in which the knife edges were very carefully ground, the first microchemical balance, which he sent to F. Pregl with the words : " This balance represents the best which I am capable of doing, and I hope that if it reaches you undamaged it will satisfy your tests." Without alteration of the essential parts, the sensitiveness of the balance had been increased tenfold by the improved grinding of the knife edges.

It is to W. Kuhlmann's definite credit to have constructed the first microchemical balance, with which F. Pregl was enabled to take with confidence the great step from the centigramme process to the milligramme process. The balance is called " microchemical balance," because the expression " microbalance " was already in use for the type of balance first employed by Nernst.

Construction and Principle of the Microchemical Balance

Kuhlmann's Microchemical Balance

(Fig. 1)

This microanalytical balance, with a beam only 70 mm. in length and a maximum load of 20 gm., has a constant sensitiveness

whether loaded or unloaded. This unusual property is due, on the one hand, to the circumstance that the three knife-blades are perfectly straight, lie in one plane, and are parallel to one another, and on the other hand, to the rigid construction of the beam, which shows no detectable distortion even at the maximum load. The balance is always provided with a rider adjustment carrying a lens, a counterpoised sliding front, hooks for carrying absorption apparatus on the left balance pan, and finally with a small screw for zero-point adjustment.

As a consequence of the improvement of the grinding of the knife edges, due to W. Kuhlmann, combined with the determination by F. Pregl of all the necessary precautions, it appears to be easily
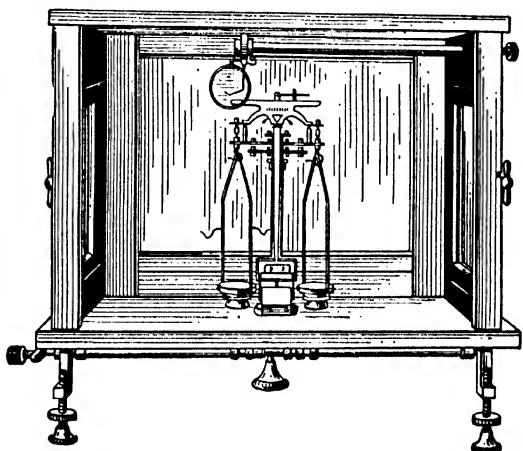
Fig. 1.—W. Kuhlmann's microchemical balance (Model 19b).

possible to obtain, with this instrument, an accuracy of $\pm$ 0·001 mgm. on a weight of 20 gm., that is, a sensitiveness of 10^{-7}. Of course, on 20 gm., the absolute determination of weight with this accuracy is affected by unavoidable factors. Since, however, microanalysis deals almost entirely with much lighter objects, and changes of a few milligrams in the weight of these are determined by difference, the stated limit of accuracy, $\pm$ 1γ, can be attained in the circumstances. It is perhaps not too much to say that this balance represents the limit of possible achievement in the construction of balances of precision.

With careful use the sensitiveness of the microchemical balance is preserved for years. Symptoms of ageing only appear after long and frequent use, and are shown by more rapid " fatigue " and also by reduction of the sensitiveness (by, at most, one-half). It is

obvious that careless handling would lead to these results more quickly.

The microchemical balance has a wider range of use than any other balances known to-day which are sensitive to differences of weight of 0·001 to 0·002 mgm., for objects of any shape, and of weight up to 20 gm., can be weighed so long as they can be stood, laid, or hung on to the balance pan.

The rider scale consists of 100 uniform notches, cut by a dividing engine in such a manner that the rider is forced into the lowest point of the notch, particularly if it is caused to oscillate by a slight push to one side. The rider weighs 5 mgm. The balance is so constructed that it is in equilibrium when unloaded and when the 5 mgm. rider is in the first notch on the left, over the left pan suspension. A displacement of the rider to the one hundredth notch, which is over the right pan suspension, corresponds to a load of 10 mgm. in the right pan. A rider displacement of 10 notches, therefore, corresponds to a change in load of 1 mgm., and thus the numerals stamped into the beam below each tenth notch indicate whole 'milligrams. These numerals start from 0 below the first notch and increase continuously to the right; therefore a displacement of the rider by one notch to the right corresponds to an increase of load of 0·1 mgm. on the right pan of the balance. This corresponds to an alteration of the equilibrium position of ten divisions on the pointer scale which is magnified by the use of the mirror. Also, one division of the pointer scale corresponds to 0·01 mgm., and as the oscillations of the swinging pointer can be read to one-tenth of a division after very little practice, it follows that, if all the necessary conditions are observed, one can weigh with an accuracy of $\pm$ 0·001 mgm. $=$ $\pm$ 1 γ. Difficulties in reading only occur with observers with abnormal eyesight, particularly with those who are astigmatic or seriously myopic, especially if these defects are not properly corrected. There is also the futher disadvantage that such observers tend to bring their heads too near to the balance and influence the regularity of the oscillations by their breathing and by the heat of their bodies. The use of a telescopic lens (C. Zeiss, Jena) is helpful in such cases.

P. Bunge's Microchemical Balance

(Fig. 2)

This, like Kuhlmann's balance, is developed from the first assay balance and constructed on the same principle. Only the differences and improvements will therefore be considered. Three different models were available for my observations, on which over 30,000

weighings have been carried out.   From the time of erection until
to-day, the balances have brilliantly stood tests on sensitiveness, in
the unloaded state and with load up to 25 gm., with the necessary
accuracy (0·001 mg.).   With two models, which have been in daily
use for five years, fatigue phenomena have not yet been observed,
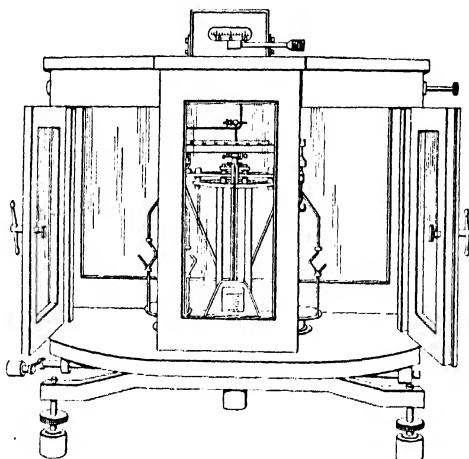
Fɪɢ. 2.—P. Bunge's microchemical balance, in a hexagonal case.

e.g., the sensitiveness after is 100, as before.   The maximum load is
30 gm. ;   the beam of the balance is 140 mm. long.   The slower
oscillation connected with it is unimportant, for the weighings are
only made after equilibrium has been reached ;   " time " weighings
are excepted, which are therefore begun some seconds earlier.   The
distance between individual notches is twice as great as in Kuhl-
mann's balance.   Hence, on the one hand, a lens for
the rider is no longer necessary, and, on the other hand,
according to investigations by E. Schwarz-Bergkampf,[1]
lower " horizontal " errors are caused by inaccurate
placing of the rider.   The shape of the rider (Fig. 3)
and the deeper cutting of the notches promotes vertical
placing of the rider.   C. Weygand[2] has already empha-
sised the fact that the more experienced experimenter is less exposed
to the error of incorrect " in-riding " which occurs occasionally,
yet it is an advance if this error can be avoided on the whole.
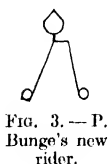
Fɪɢ. 3. — P.
Bunge's new
rider.

The protection for suspensions and beam shown in Fig. 4 is very
important in balances provided for students.   It prevents the beam

[1] E. Schwarz-Bergkampf : *Z. anal. Chem.*, **69**, 321 (1926).
[2] C. Weygand : " Quantitative Analytical Micromethods of Organic Chemistry."
Leipzig : Akademische Verlags-Gesellschaft. m. b. H., 1931.

from slipping on its point of support owing to the dropping of the suspensions, which is seen occasionally in collisions with the absorption apparatus.

More recently, P. Bunge has erected the balance in a very practical hexagonal case (Fig. 2). The two large doors compel the weighing to be done according to F. Pregl's instructions, using the right hand for the right balance pan and the left for the left. The rider scale and the suspensions are readily seen through the fixed front glass wall. At the level of the eye, in the middle on the front wall of the case, a reading giving the position of the pointer can be observed by a projection arrangement. (By means of a special source of light,[1] the transparent scale placed at the pointer is projected on to a translucent strip of glass, which is provided with a movable index-line.) Since 0·001 mgm. can easily be estimated from a distance of 50 to 60 cm. in front of the balance, and, moreover, the macro-scale at the foot of the pillar, for rough weighing with the rider, can be observed from the same distance through reflection in two mirrors, it is no longer necessary to draw near during intervals in the experiment when the arrestment is liberated, and the influence of the warmth of the body on temperature is practically excluded.
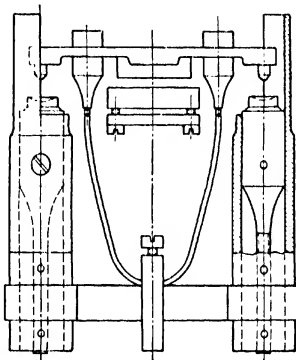
Fig. 4. —Protection for suspension and beam.

Of the different microchemical balances on the market, my experience of that of Starke and Kammerer has been very good.[2] It is supplied with two interchangeable degrees of sensitiveness, by which means the discovery of the position of the rider is facilitated, and, not least, the smaller oscillation of the beam in the rough weighing contributes to the sparing of the knife edges. The readings are effected with a telescopic lens placed in the case in front of the scale, which is preferable to the use of a concave mirror, especially for those who are markedly ametropic.[3]

P. Bunge's Aperiodic Rapid Balance

The oscillations are damped down with air by means of two concentric cylinders and the deflections are read, with the help of a

[1] The light is only switched on for the reading. A 4-volt accumulator lasts two to three months.
[2] *Cf.* also L. Fuchs : *Mikrochem.*, **10**, 456 (1932).
[3] To-day balances with telescopic or lens readings are obtainable from all firms.

collimation-telescope, on a macro-scale attached laterally and similar
to that of Kuhlmann's microchemical balance. The estimation of
0·001 mgm. is possible with greater accuracy on the stationary scale.
It is very important that the balance should always be well levelled,
otherwise, through reciprocal touching of the cylinders, braking occurs
all too readily and injures the readings very much. The bottom,
back wall, and both side walls of the case are made of aluminium,
for thermal and electrostatic equalisation. In my experience, this
is only an advance on the ordinary balance cases, if the balance is
again surrounded by a protecting case, because as the metal walls
equalise differences of temperature inside the balance, they are also
subject to temperature changes of the balance room.

Kuhlmann's Aperiodic Microchemical Balance

The form is similar to that of the microchemical balance
described under (1). Equilibrium is obtained by first employing
weights and then the 5 mgm. rider to the nearest tenth of a milligram.
Reading to 0·01 and 0·001 mgm. is now done directly, with the
telescopic lens, on the scale which extends from left to right and is.
divided into 100 parts ; one division represents 0·001 mgm., and
ten divisions 0·01 mgm. The essential difference between this
instrument and the balance described under (1) lies in the fact that
here the determination is not made on the reversal points of the
swinging pointer, but on its position of stationary equilibrium. A
braking device is provided, which is very easily applied and with-
drawn, by means of which the rapidly oscillating balance is so
retarded that the position of equilibrium is very quickly attained.
The essential feature of this braking device is a small, short beam,
with an excess weight of 1 mgm. By means of this a flat-ground
agate plate is pressed against a highly-polished rotary member
which is attached to the balance beam in such a way that its axis is
in line with the middle knife edge.

The telescope is now placed in the centre above the balance case,
in a very convenient position for the observer, and is divided in such
a manner that it in no way interferes with the use of the sliding
front. The light-track has been greatly lengthened by the interpola-
tion of two prisms, and great magnification of the pointer reflections
has thus been attained. As very small deflections can thus be
observed, it is especially important that the balance supports should
be free from vibration.

The actual weighing operation is extremely simple. Equilibrium
is first restored to within 0·1 mgm. in the usual manner by adding
weights and adjusting the 5 mgm. rider, during which working of the
pointer is observed with the help of a millimetre scale, 1 mm. of

which corresponds to 0·1 mgm. The balance is then arrested, the lever-brake applied by liberating the suspended lateral weight and then the arrestment again liberated. By means of the telescope one may now observe the indicator move into the new position of equilibrium and come to rest there or in the vicinity. When the lateral weight is then lifted and the brake thus removed, the indicator moves further through a few divisions and finally comes to rest after the brake has been repeatedly applied and removed.

From this description it will be apparent that the manipulation of this balance is decidedly different from the usual procedure and requires some little practice. The balance is, however, particularly convenient for certain weighing operations, *e.g.*, for weighing substances into boats, weighing absorption tubes, halogen filter tubes, etc., as by observing the course of the image through the telescope it is easy to recognise when temperature equilibrium between the object and the air in the balance case has been attained. Numerous such investigations have confirmed the correctness of the time intervals indicated in the first edition of this book. Unfortunately, the anticipation that this balance might enable the accuracy of weighing to be multiplied tenfold, compared with the one previously described, has not been fulfilled. In spite of the great advantage, which will appeal to many, of being able to observe the position of a stationary line of light and not the turning points of a rapidly swinging pointer, the model described in the first place will continue, as before, to be the chosen instrument.

The Ultra-microchemical Balance

For Pregl's micromethod, it is not necessary to weigh to a greater degree of accuracy than $\pm$ 1 γ, so long as the differences between the objects to be weighed—*i.e.*, absorption apparatus for the carbon and hydrogen determinations—and material weighed out are still proportionally great. For the highest refinement of working (using 1 mgm. of material) exists, however—*e.g.*, for weighing light apparatus (determination of residues, electrolysis)—the need of an instrument which is ten times more accurate, in which the thousandth part of a milligram is read, not estimated, and only ten thousandths are estimated.

According to E. Schwarz-Bergkampf,[1] the intervals between the separate notches, which are measured in a horizontal microscope, amount to 0·006 mm. Thus, at the worst, for a distance of 0·7 mm. between the notches, the error in weighing is limited to 1 per cent. Whilst for the usual microchemical balances this variation lies

[1] E. Schwarz-Bergkampf : *Z. anal. Chem.*, **69**, 321 (1926).

within the experimental error, it would affect results on an ultra-microbalance to the extent of 10 per cent.

It is entirely otherwise if, instead of the 5 mgm. rider, a 0·5 mgm. rider is used for constant intervals between the notches. The error of position, indeed, again amounts to 1 per cent., but with respect to the 0·5 mgm. rider, this is still only 0·0001 mgm. = 0·1 γ.

On this principle, W. Kuhlmann has brought an ultra-micro-chemical balance with two rider scales into business. With this balance, by taking various precautions, C. Weygand [1] has succeeded in carrying out determinations of residues on substance weighing less than 1 mgm., with an accuracy of $\frac{1}{4}\gamma$.

Besides the usual equal-armed balances, there are also important, more sensitive instruments, which, however, in consequence of their small loading capacity, can scarcely be used as long as the objects to be weighed are still relatively heavy in comparison with the amount of material used. Extremely fine balances, which, with a maximum load of 0·3 gm., indicate differences in weight of 0·0001 mgm. = 0·1 γ, have been fully described by F. Emich.[2]

In recent years, E. Wiesenberger,[3] in F. Emich's laboratory, has been able to improve Knut Angström's electrometric compensation balance to a high degree, and to carry out determinations of residues in small platinum dishes and electrolysis by the Gamma process with greater accuracy.

Weighing with the Microchemical Balance

It is advisable to count deflections (the middle point of the scale counting as zero) by considering the tenth part of a division as unity ; for instance, a deflection of 2·7 scale divisions to the right is described as " 27 right " and a following deflection of 3·4 scale divisions to the left as " 34 left." The mean deflection from the zero point is in this case " 7 left," that is, one must subtract 0·007 mgm. from the sum of the weights on the right hand pan and the weight corresponding to the rider reading. If the readings were " 39 right " and " 30 left," then 0·009 mgm. would be added to the above sum.

The sensitivity of the balance, however, is not considered accurate for 100 : also fatigue is caused more by the larger than by the smaller deflections. It is therefore made a rule not to allow the pointer to swing more than six scale divisions and never to read larger differences than 50 units. For larger differences, the rider is placed in the next notch. If the pointer swings out too far, it is

[1] C. Weygand : " Quantitative Analytical Micromethods of Organic Chemistry." Leipzig : Akademische Verlags-Gesellschaft. m. b. H., 1931.

[2] F. Emich : Abderhalden, "Handbuch der biologisch Arbeitsmethoden," Section I., Part 3, p. 183.

[3] E. Wiesenberger : *Mikrochem.*, **10,** 10 (1932).

advisable to stop it while the pointer swings through the zero and to bring it into motion again by releasing the arrestment more slowly.

It is also advisable not to calculate the difference between two successive swings mentally, but to note down the deflections themselves. The calculation distracts many people from observation of the swings, also many tend too much to find the same differences subjectively in estimating successive swings. Since the differences between separate whole oscillations only cause very small and regular reduction of the swing, comparison of the differences on paper is more objective than mental calculation. This method of observing the swing enables one to test the accuracy of the weighing at any time ; for a deflection to the right side of the scale is to be added to the weight, and to the left must be subtracted from it. If we therefore displace the rider, after a positive deflection has been obtained, by one notch to the right, the deflection will become negative and to the left. This, when added to the former positive deflection, should result in a value of 100, if 100 is the sensitiveness of the balance. In this manner one can also check the correct adjustment of the sensitiveness of the balance, both when unloaded and at maximum load, without difficulty.

The Erection of the Balance

Suitable support of the balance is particularly important. It has been found well to use marble slabs (3-4 cm. thick), supported on iron bearers which are mounted in the wall. Sheets of lead are placed between the marble slabs and the iron bearers.

Moreover, the feet of the balance are in vibration absorbers with rubber discs, which are supplied with the balance.

Especially in buildings of concrete, vibrations from machines or centrifuges pass through the walls, from where they are transmitted through the iron bearers to the balance. Such disturbances are completely avoided by means of a balance table, which stands away from the wall, the legs of which stand on two heavy concrete plates, with bases of masonry, 25 cm. broad and the depth of the table (60 cm.). Between the floor and the bases, as between marble slabs and supports, several sheets of lead, 1·5-2 mm. thick, are placed. By this means balances are completely protected from vibrations of every kind. Frequently sheets of cork or rubber are recommended instead of lead as insulating material. A warning is given against this because, instead of a vibration-absorbing table-top insensitive to pressure, we should have one which reacted to any experimental pressure—*e.g.*, by one-sided loading (due to desiccators) alteration of the zero of the balance would be produced.

In choosing a locality for the erection of the balance, the lighting and heating arrangements of the room are often more important than freedom from vibration. Whilst the microchemical balance is little affected, if at all, by the passage of a tramcar in the vicinity, it is very sensitive to air currents within the balance case. Therefore it is inadvisable to mount the balance in front of a wall in which there is a heating unit. It must not be placed near a stove, but is best placed against the opposite wall. The balance must never be exposed to direct sunshine, and one should avoid the presence of a lighting unit in the immediate neighbourhood. All the above-mentioned influences cause deflections of the zero point which, if they are small and tend to a constant value, such as the change from daylight to artificial light, do not affect the accuracy of the weighings, so far as we know. Frosted electric lamps, placed 85 cm. above the top of the table and immediately above the balance, are most suitable for the artificial lighting of balances. If one is unable to find a place for the erection of the balance which is sufficiently favourable completely to avoid variations of the zero point, these must be taken into account, more particularly in the case of drying operations, which may last for hours or days.

Even in balance rooms in which all the above requirements have been taken into account, a larger or smaller alteration in the zero is obtained during the course of a day's work. The position of the zero in all microchemical balances depends on the temperature. Since it is very seldom possible to keep the temperature of the balance-room constant to $\pm 0.5°$ C., a determination of the zero point must be made for all weighings which are spread over more than an hour, as follows :

1. The zero point deflection is expressed by the amount in thousandths of milligrams by which the unloaded balance differs in successive readings, the second of which has taken place some time after the first. It is considered to be positive if the alteration is from the left to the right and negative in the contrary case.

2. The true weight is found by adding the zero deflection with the reversed sign, that is, a positive zero point deflection must be subtracted from the weight which is found and a negative value must be added.

One may readily demonstrate the effect of air currents inside the balance case by two experiments. If one places a body of temperature only slightly higher or lower than that of the surroundings, for instance a block of copper, near the left pan of the balance, one finds after a short interval, in the first case a zero point deflection to the left, and in the other case a similar deflection to the right. The zero point returns to normal after removing the disturbing object.

On touching the side door of the closed balance case with the palm of the hand a zero point deflection is also observed. On then opening the balance front and both side doors the zero point is restored. It follows, that during weighing no objects (weight forceps, copper block, and so forth) which are not already there should be left in the balance case, and, on the other hand, that objects which are always necessarily used when weighing (weights, counterpoises, counterpoised flasks, together with the necessary supply of shot for the latter), should be kept permanently in the balance case, where, moreover, they are best protected from dust.

Since the general introduction of weighing with a counterpoise in 1912 only a very few of the weights which are provided with the microchemical balance have been used, namely, the 1 gm., 0·5 gm., 50 mgm., two 20 mgm., and the 10 mgm. weights. The accuracy of these must occasionally be tested, as after use for some years they show a remarkable tendency to become rather heavier. By brushing them, washing them with water and alcohol, and finally polishing them carefully with a cloth, or in extreme cases with rough paper, they are restored to their original exact correspondence with the rider readings. The rider, which was formerly made of thin platinum wire, also becomes heavier in the course of time, and becomes strongly discoloured ; the small weights then appear to be too light. Such riders can be restored to equality with the 10 mgm. weight by careful washing in dilute potassium cyanide solution, followed by gentle friction between the finger-tips. W. Kuhlmann now makes his 5 mgm. riders of aluminium wire. These are of larger volume, do not become discoloured, and remain, in general, in good accordance with the other weights. Gold riders especially, however, as given with the Bunge balances, remain of constant weight for years. The slight increases in weight appearing later are easily removed, as above, by washing and polishing, if necessary, with paper. Decrease in the weight very seldom occurs. In such cases a new rider is ordered, which is very cheap to-day, and its accuracy is tested against the set of weights.

For this, the zero of the balance is first determined with the rider in notch 0. To test it against the 10 mg. weight, this is placed on the left balance pan and the rider in notch 100. Whilst the rider is left in the same notch and the 10 mg. weight placed on the right balance pan, the 20 mg. weights are checked successively on the left pan. Then the 50 mg. weight is placed on the left pan and tested against both 20 mg. weights on the right and the rider in notch 100.

The weights are best kept in the balance case in a small flat dish, the bottom of which is lined with black velvet. For reasons already referred to, one should not keep the hand in the neighbourhood of

the balance for longer than is absolutely necessary for opening and closing the arrestment, and should make it a rule to leave the balance case open before beginning a series of weighings in order to ensure complete elimination of any possible differences of temperature and moisture between the air within and without the case. We refer to this as " acclimatisation."

In 1926, E. Schwarz-Bergkampf,[1] with F. Emich, made investigations on the precautions necessary in weighing and was thereby able to make valuable additional determinations of the errors which arise if the temperature of the body weighed differs from that of the balance room, or if two weighings separated by a time interval are carried out at different temperatures of the room, or if the rider deviates by definite amounts from the vertical. According to these observations, a balance room temperature as constant as possible, with daily fluctuations of less than 1° C., ensures a constant zero. These determinations confirm the standard conditions for weighing which have been established for twenty years and which were laid down as absolutely necessary in the first edition of this book.

It is very important for the satisfactory working of each balance that the directions for mounting, which are sent with it, should be followed most accurately, as otherwise it is easy for the enthusiastic beginner to cause serious damage.

Cleaning the Balance

It is necessary to clean the balance thoroughly from time to time, particularly when the arresting contacts adhere and the pointer (beam) is caught on one side on releasing the arrestment. Uncleanly manipulation when erecting the newly arrived balance may cause this to occur for a considerable time.

The doors are opened and, in this order, the rider carrier, pans, suspensions, and beam are removed. The three last-named parts are laid consecutively on the rider carrier and the floor plate is well cleaned with moist gauze, then the balance pans and suspensions are rubbed with chamois leather which has been washed free from fat and acid and well dried. The notched rider scale of the beam is carefully brushed with marten-hair brushes and all arresting contacts (on beam, suspensions, and pillar arrestments) are rubbed thoroughly with dry chamois leather.

In one case of apparently irremovable stickiness F. Pregl finally achieved success by painting the pans and the hemispherical arrests with a paste of previously ignited talc and alcohol, and rubbing off the former with chamois leather after drying.

[1] E. Schwarz-Bergkampf : *Z. anal. Chem.*, **69**, 321 (1926).

Finally one cleans the knife edges and the corresponding supports, also with dry chamois leather. This operation is best conducted with the help of a watchmaker's lens, which is indispensable both for mounting the balance and for other work and should always be in the pocket of the microanalyst. The balance is now re-erected and tested to see if the zero point has been altered. This is always the case if one has touched the vane. If the zero point deflection is large, it is approximately corrected by a small screw on the vane. Handling of the screw with the fingers causes an additional deflection of the zero point which lasts for some time. It is therefore particularly desirable, in correcting small variations, to turn the screw with forceps, in order to avoid such warming of the vane.[1] The final adjustment of the zero point, which is concerned with the last 0·01 to 0·02 mgm., is best effected by means of the two levelling screws of the case.

Very fine hairs under the central knife edge, under the suspension knife edges, or on the end of the pointer may be very troublesome through obstruction of the oscillation. Such faults are liable not to be detected and remedied by the inexperienced if attention is not directed to them.

Whilst the zero point of the balance is usually altered on dismounting and cleaning, as one can hardly avoid touching the vane which adjusts this, the sensitiveness is never changed by these operations if they are conducted with reasonable care. The two vertically adjusted counter-screws, which control the sensitiveness, are screwed so tightly that their position cannot be changed by ordinary handling, since, at the suggestion of F. Pregl, the vane for the zero adjustment is no longer attached between these two counter-screws but independently to the beam. One must, however, distinguish between temporary alterations of the sensitiveness, due to warming the vertical screw for some time with the fingers, and permanent alterations which can only be due to serious misuse.

Counterpoises

It is advisable to prepare suitable counterpoises for weighing objects which are repeatedly used (boats used in the carbon determination, platinum crucibles, Neubauer crucibles, filter tubes, absorption apparatus). Such counterpoises have been used in technical laboratories for a long time. One prepares a counterpoise (Fig. 5 a) for the platinum boat from aluminium wire 2 mm. thick, which is

[1] For years W. Kuhlmann has provided a light vane with light wings instead of the difficult screw mentioned. This vane can readily be turned with the open forceps, so that the zero adjustment can be made particularly easily without warming. P. Bunge equips the balances with a device which permits the wing screw to be moved from outside by means of a forked rod.

bent twice so that its three portions correspond to three edges of a tetrahedron. This counterpoise is adjusted by filing until, when balanced against the boat, the rider is in equilibrium in the neighbourhood of the first milligram. Hence, in weighing substances the rider alone need be used, without additional weights.

So that it need not take long to find the appropriate counterpoise when weighing, the counterpoise is stamped with the number of the small desiccator in which the boat is always kept.

Small counterpoises of fine aluminium wire, weighing about 5 mgm., are very convenient, as they enable one quickly to adjust the position of the rider, from beyond the notch 50, to within the first milligram of the rider scale.

For counterpoising heavier objects (absorption apparatus and filter tubes), thin-walled glass flasks (Fig. 5 *b*) are used, which can be obtained from W.

Fig. 5.—*a*. Aluminium counterpoise for the boat. *b*. Counterpoise flask with shot. (Actual size.)

Kuhlmann and P. Haack, marked with consecutive figures. A special counterpoise flask may be prepared for each· piece of apparatus with the help of the necessary arrangement of small lead shot (Shot No. 15).

It is advisable, in preparing such a counterpoise, first to load the flask on the right balance pan with coarse shot and to place beside it a 50 or 100 mgm. weight. As soon as the balance turns to the left one replaces the weight by finer shot until the balance again swings to the left ; one then removes one shot and examines whether the rider must be brought beyond the number 5. If this is the case, then finer shot is placed on the right pan ; if the result is still positive, the shot is brought into the flask. This is the quickest way of effecting the adjustment, and the small amount of work necessitated by preparing these counterpoises is amply repaid during subsequent weighings.

In recent years nitrogen weighing tubes with long or short glass handles have come into general use instead of tubes with aluminium handles. They are used for all weighings except those in boats and capillaries. The glass weighing tubes (Fig. 24 *a*, *b*) are so made, that their weight only differs from 0·5 gm. or 1 gm. by a tenth of a milligram. The rider alone is then necessary for weighing, and space is saved in the balance, because 500 mgm. and 1 gm. from the set of weights are used as counterpoises.

B. THE DETERMINATION OF THE ELEMENTS

I. F. Pregl's Method for the Determination of Carbon and Hydrogen

History and Principle of the Method

The highly important micromethod undertaken by F. Pregl in 1910 underwent many alterations and fundamental changes until 1916. Originally the gases escaping from the absorption train were collected in a mercury gas-holder and then passed once more through the red-hot tube. Later, by using a longer tube, and especially by means of an efficient oxidising charge, it was possible to avoid this trouble.

The experience acquired by this work with the mercury gas-holder established the importance of the pressure and speed relations in the whole system, and especially within the absorption train.

To control the necessary conditions with certainty was made possible in excellent ways through the combined effect of pressure regulators, choking plugs, and Mariotte flasks. From numerous painstaking combustions of substances containing halogen, nitrogen, sulphur, and arsenic, F. Pregl developed the so-called " universal " filling, which consists of copper oxide and lead chromate mixture, lead peroxide, and metallic silver ; this filling enables us to-day to burn any substance whatever correctly. It was found that, in spite of its many objectionable qualities, lead peroxide was an absolutely reliable absorbing agent for higher oxides of nitrogen. Halogens and sulphur were retained before these by metallic silver and lead chromate.

The unique value of this method for the determination of carbon and hydrogen in the smallest amounts of organic substances, is due to the fact that each separate condition and each modification taken into consideration has always been checked by a large number of blank experiments and by analyses of known substances, so that ultimately a process was obtained, the accuracy and reliability of which is still unsurpassed. It is an analytical method, without which the tremendous progress made in the two last decades, especially in physiological chemistry, could not be imagined.

Since the publication, in 1912, of F. Pregl's method, a large number of works by his pupils and other authors have been published, which treat in detail the troubles which appear temporarily, and also those which extend over a longer time. Since the appearance of the third edition of this book, particularly thorough and careful studies have been made of sources of error which are to be reckoned either temporary or permanent according to circumstances. These have

been made in particular by J. Lindner,[1] M. Boetius,[2] A. Friedrich,[3] and B. Flaschenträger.[4]

These authors have collected numerical data for nearly all such occurrences and phenomena, on the basis of which their influence on the total result can be reckoned, and from this it can be seen whether a source of error is to be considered or to be disregarded.

Whoever observes exactly the principles and directions described later will, in any case, with practice and experience, be able to reduce all three groups of sources of error adduced by M. Boetius to a minimum which lies within the analytical accuracy of $\pm$ 0·3 per cent. for carbon and $\pm$ 0·3 per cent. for hydrogen, and will be convinced, with B. Flaschenträger, that microanalysis need not always be devised afresh. The sources of error are :—

1. Errors of the apparatus.

2. Insufficient purity of the reagents and inadequacy of the auxiliary material.

3. Incorrect manipulation of the apparatus.

In the Kaiser-Wilhelm-Institut, Heidelberg, there has been no cause to alter the Pregl method in principle in any direction, nor to replace it by another. It has never shown disturbances worth mentioning, even after interruptions which last for days and weeks and which might cause one to lose confidence in it. I must state that in about 5,000 different substances I have not met with any which could not be analysed with the required accuracy of $\pm$ 0·2 per cent. for carbon and hydrogen.

In this connection it may be mentioned that, according to a communication from O. Leschhorn, F. Pregl's principle has also proved excellent in the Institute of Professor H. Fischer, Munich, for the half-micro-method, on the following weights of material : For nitrogen, 5-10 mgm., for carbon and hydrogen, 15 to 20 mgm. It was therefore introduced into the curriculum. This has the advantage that for those who are conversant with the principle of Pregl's method, the step from half-micromethods to micromethods in the given cases presents no difficulty.

Oxygen and Air (Purification of the Gases)

To-day pure oxygen from liquid air is almost always used.[5] It is either drawn directly from steel bottles (bombs) or from gas-

[1] J. Lindner : *Ber. dtsch. Chem. Ges.*, **59**, 2561, 2806 (1926); **63**, 949, 1123, 1396, 1672 (1930).

[2] M. Boetius : On the sources of error in F. Pregl's method for the determination of carbon and hydrogen.

[3] A. Friedrich : *Z. angew. Chem.*, **36**, 481 (1923).

[4] B. Flaschenträger : *Z. angew. Chem.*, **39**, 717 (1926).

[5] Note to translation of 3rd edition : " The translator has found that commercial oxygen produced by the fractionation of liquid air may, in certain circumstances,

holders (bottles) after filling. Gasholders which deliver the gases at constant pressure have been specified by H. O. Hohl [1] and J. Lindner. [2]

The air, of which about 80 c.c. are drawn through the apparatus and absorption tubes for one analysis, must be completely free from carbon dioxide and organic constituents. For freeing from carbon dioxide, leading it through the U-tube with bubble-counter usually suffices. The organic vapours contained in the air of the laboratory are avoided by filling the gasholder in the open air, or by drawing in pure air from outside. If, however, gasholders stand for some time in the laboratory, vapours of organic solvents may be partly absorbed by the sealing liquid and may enter with this into the gasholder and, finally, the apparatus.

This source of error, which is indeed only small, can be avoided if oxygen and air are used directly from the bomb, with the aid of a (needle) reducing valve [3] for regulating pressure. The air should not be obtained from liquid air, but from pure atmospheric air by compression at 160–180 atm.

The taking of the gases directly from steel bottles has been approved for years by the Kaiser-Wilhelm Institut. [4] A 3-litre bomb in daily use lasts more than a year ; also one has exactly the same gases at one's disposal, and the cost is less than for gasholders of constant pressure.

A blank test must be made on the gases of new bombs (p. 62). The gases are irreproachable, if in the blank test the calcium chloride tube shows an increase in weight of less than 0·05 mgm. and the soda-lime tube, one not more than 0·02 mgm.

If gases which are not open to criticism cannot be obtained even in steel bottles, e.g., if the air gasholder must be filled in factory yards, then increases in weight of the absorption apparatus would appear in the blank experiment.

In such cases the gases must be freed from vapours containing carbon and hydrogen, for which the " catalyser-pipe " (Fig. 6) of F. Böck and K. Beaucourt [5] is inserted between the three-way cock and the bubble-counter. This consists of a glass tube filled with platinised asbestos, which is electrically heated to 600° C. Fused on to the heating unit is a cooling device, which cools the

carry minimal amounts of organic matter which seem to be due to the slight vapour tension of the oil used in the compressors, so that, in his opinion, the above statement must be accepted with a certain degree of reservation.—E. F."

    [1] H. O. Hohl : *Mikrochem.*, **13,** 189 (1933).
    [2] J. Lindner : *Mikrochem.*, **13,** 313 (1933).
    [3] Obtainable from A. Hofer, Mülheim (Ruhr).
    [4] According to private communications from Dr. E. Wiedemann, this type of gasholder is much approved of in the micro-laboratory of the Chemischen Fabrik, formerly Sandoz, Basle.
    [5] F. Böck and K. Beaucourt : *Mikrochem.*, **6,** 133 (1928).

gases to room temperature before they enter the next rubber connection. The water and carbon dioxide thus formed are retained in the U-tube which follows.

The catalyser-pipe should only be used when the blank test on the gases indicates an increase in weight; it should not be connected merely as a precaution, since the apparatus thereby only becomes more complicated and the junctions might give rise to a new error

The Delivery Tubing. This, which is used to connect up all parts of the apparatus, from the gasholder to the combustion tube, has been exhaustively investigated by F. Pregl and, later, by F. Böck and K. Beaucourt,[1] A. Friedrich,[2] J. Lindner,[3] and M. Boetius.[4] To-day, through appropriate preliminary treatment, it is scarcely open to question as a serious source of error. Faulty increases in weight in blank tests have been established by F. Pregl only for insufficiently matured tubing, e.g., new tubing which still gives up organic solvents and for tubing through which organic gas (illuminating gas) has previously been passed.[5] The tubing was therefore submitted to an artificial ageing process, in which air was aspirated through it in a drying oven (100°–110° C.) for one hour with a water pump. According to A. Friedrich[6] this type of pre-treatment cannot be used with certainty for all grades of rubber; therefore in the third edition of this book the following " artificial ageing " according to A. Friedrich was recommended by F. Pregl :
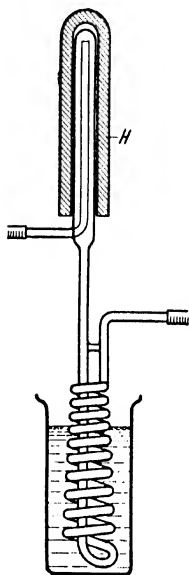
Fig. 6. — Catalyser-pipe of F. Böck and K. Beaucourt. *H.* Electric heating unit.

" The new indiarubber tubes of 3·5–4 mm. bore and 1–1·2 mm. thickness were kept in 40–50 per cent. caustic potash solution on a water-bath at 60° C. for two hours, then washed well with water, and steam was passed through them for two hours. Artificially aged tubing may be bought from the firm of P. Haack. It is advisable to steam out the tubes of the apparatus at least twice a year. For all longer connections which may be necessary the use of well-joined glass or lead tubes is recommended."

[1] F. Böck and K. Beaucourt : *Mikrochem.*, **6**, 133 (1928).
[2] A. Friedrich : *Z. angew. Chem.*, **36**, 481 (1923).
[3] J. Lindner : *Ber. dtsch. chem. Ges.*, **60**, 124 (1927).
[4] M. Boetius : " The Sources of Error in the Microanalytical Determination of Carbon and Hydrogen according to F. Pregl's Method." Berlin : Verlag Chemie, 1931.
[5] *Cf.* p. 20. Admission of organic vapours from the sealing liquid of the gas-holder.
[6] A. Friedrich : *Z. angew. Chem.*, **36**, 481 (1923).

Pressure Regulators with Precision Pinchcocks and Three-way Cocks

For the regulation of the gas currents screw pinchcocks on the tubing have hitherto served almost exclusively. With the increasing
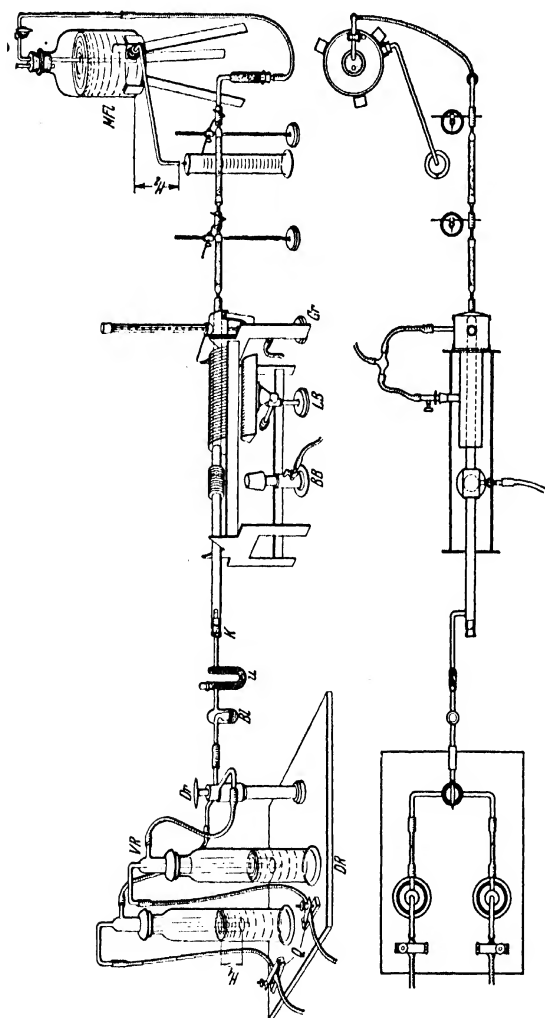
FIG. 7.—General view of apparatus for determination of carbon and hydrogen in elevation and plan. ($\frac{1}{20}$ actual size.) *DR*, Pressure regulator. *VR*, Adjustable tube of regulating gasholder. *H_1*, Pressure head. *Q*, Precision pinchcock. *Dr*, Three-way cock. *u*, U-tube with *Bl* (bubble-counter). *K*, Rubber stopper. *BB*, Movable burner. *LB*, Tube burner. *Gr*, Heating mortar. *MFl*, Mariotte flask. *H_2*, Suction head.

improvement of the method, however, it became urgently necessary to adopt safety devices which would render impossible an unexpected increase in the pressure and consequent velocity as a result

of careless handling of the pinchcock. In 1912, the early realisation of the fact that for a successful analysis a minimum time of contact of the combustible vapours with the individual portions of the tube filling is essential, rendered it necessary to ensure that equal quantities of combustible vapours passed through the cross section of the glowing tube in equal times.

Such control was attained in a very perfect manner by the pressure regulator (DR, Fig. 7) which is mounted on a board. This comprises essentially two bell gasholders, of which one is used for introducing the air and the other for the oxygen. Each consists of a glass bottle about 240 mm. high and about 60 mm. external diameter, which is about two-thirds filled with 5 per cent. caustic soda solution and the mouth of which is closed with a wooden cap. The bell of the gasholder passes through a central perforation of the wooden cap and is adjustably held in place by three metallic springs. This bell consists of a wide glass tube, 20 mm. in diameter and 200 mm. long, within which a narrow glass tube, 3–4 mm. clear width, is sealed. The lower end of the inner tube reaches 6–7 mm. beyond the open end of the wide tube. This inner tube constitutes the inlet tube for the gas and is bent twice externally at right angles and connected to the corresponding gasholder through a matured rubber tube provided with a precision pinchcock Q (Figs. 7 and 8). The gas passes out through a glass tube sealed to the side of the upper end of the bell tube, and which is connected by matured tubing with the corresponding limb of the three-way cock Dr. By turning the stopcock we are able to replace oxygen by air after the complete combustion of the material, or to cut off the supply of gas.

On passing air or oxygen into the pressure regulator the level of the liquid in the bells sinks to their lower rims and the excess of gas escapes below in small bubbles. If the inner tube were made of the same length as the surrounding cylinder, large bubbles would be formed, which would escape occasionally. The pressure of the escaping gases is determined by the difference of level H_1 (Fig. 7) of the liquid in the inner and outer vessels. Since the greatest difference H_1 can never be increased, the arrangement therefore ensures that gas pressure once set up is never exceeded. As the gas speed in the combustion tube, under the conditions present in the same, is dependent solely on the pressure, we are able definitely to adjust it by raising or lowering the movable bell-tubes, for the lower the adjustable tubes are plunged in the sealing liquid the greater is the gas pressure and the consequent gas speed, and *vice versâ*. It is obvious that, for reasons of economy, one should reduce the inflow of gas into the pressure regulator, by means of the needle

valve or the precision pinchcock, to such a degree that the necessary pressure difference is just maintained and that gas bubbles escape from the bells at intervals of at least two to three seconds. If the gases are brought directly from steel bottles, the pinchcock is not needed, for with good needle valves the speed of the bubbles can be adjusted more equally.

If one is obliged to set up the apparatus for the determination of carbon and hydrogen in a room which cools down greatly overnight—*e.g.*,

Fig. 8.—Precision pinchcock.
(½ actual size.)

rooms which are steam-heated in winter—then with long inlet tubes it may happen that the sealing liquid of the bell gasholder is sucked back into the gas inlet tube. If the liquid reaches the pinchcocks, the tubing must be removed and completely dried, so as to avoid trouble in the subsequent working of the apparatus. In such cases, according to the means approved by Dr. E. Wiedemann, the bell gasholders are developed as safety gasholders on the principle of safety wash bottles; *i.e.*, the central inlet tubes are provided with a sufficiently large bulb.
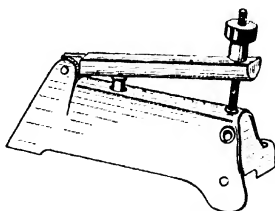
The U-tube with Bubble-counter

The gases pass from the three-way cock through an attached tube, 35–40 mm. long and 5 mm. outer diameter, into a bubble-counter (Figs. 7 and 9). As shown below, the tube is bent at right
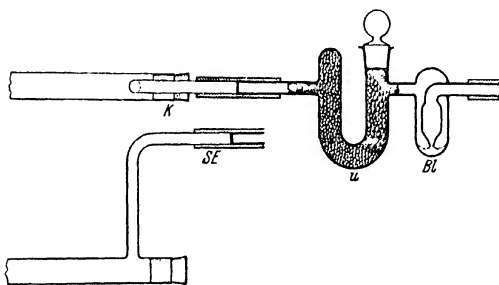
Fig. 9.—Connection of the U-tube (*u*) with bubble-counter (*Bl*) to the side-tube (*SE*) of the combustion tube, the mouth of which is closed by a cork (*K*).

angles about 8 mm. after the position of fusion into the pear-shaped jacket; then it widens into a bulb and ends in a fine tip, the diameter of which does not exceed 1 mm., which is 3–5 mm. above the bottom of the outer jacket. The gases escaping from the tip pass into the U-tube fused on to the side of the bubble-counter. This U-tube

consists of a glass tube, 120–140 mm. long and 12 mm. outside diameter, which has a ground-in stopper at the end which is attached to the bubble-counter. At the other end it is closed above the fused-in outlet tube. This outlet tube has the same external measurement as the inlet tube and is connected with the side tube of the combustion tube through rubber tubing about 50 mm. long, glass being in contact with glass. The function of the U-tube with bubble-counter is reported on with the filling (p. 39) and the calibration of the combustion tube (p. 47).

Combustion Tube and Furnace

The combustion tube consists of hard Supremax glass from Schott and Gen., Geneva. It has an external diameter of 9·5–10·5 mm. and is 500 mm. long, exclusive of the length of the neck.

The gases are not, as formerly, led in through the mouth of the tube, but a side tube[1] bent at right angles is attached about 18 mm. from the mouth, as in Fig. 9. Its external diameter is 5 mm. and its inner one is at least 2 mm. The advantage of this is that the bubble-counter need not be moved to the side with every filling of the combustion tube. Hence, three-way pieces, U-tube, and side inlet tube may be inserted with the glass in contact with glass.

At the other end, the combustion tube passes into a neck of 3·3–3·5 mm. external diameter and 2–2·5 mm. aperture. A neck 23–25 mm. long has proved best in practice ; for this length of the neck by far the best fall of temperature is situated just in this critical part of the apparatus. On the one hand, no collection of condensed water occurs, and, on the other hand, the absorption tubing is not so readily injured by the heating mortar. It is advisable to form this neck, not by drawing out the tube but by carefully sealing on a tube of the given dimensions. The end of the neck is ground even, perpendicular to the axis, first with coarse and then with fine emery or carborundum powder. The edges are rounded off in the flame without constricting the bore. It is inexpedient to replace the Supremax glass tubes by quartz tubes. First, with careful handling, the Supremax glass tube lasts for about 200–300 analyses, a number which covers the life of the universal filling, if occasionally substances containing halogen and nitrogen are burnt ; second, the quartz tube is appreciably dearer ; and third, the quartz tube is cloudy and opaque even after fifty to seventy analyses.

Before heating, the tube is protected from direct contact with the flame of the tube burner (*LB*), and from distortion, by means of a surrounding roll of fine mesh wire gauze (Fig. 10), 180 mm. long (*El*). A similar roll (*Ek*) 50 mm. long surrounds the tube in the position of the movable burner. The entire filling of the tube is heated to

[1] G. Lunde : *Biochem. Z.*, **176**, 157 (1926).
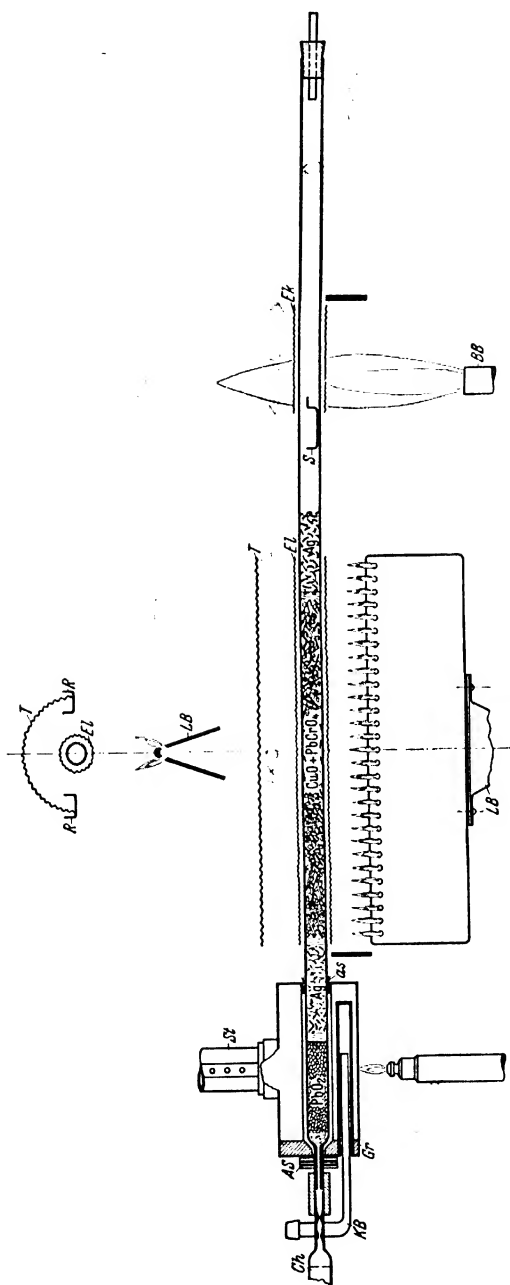
Fig. 10.—The filled combustion tube in position for analysis. (⅓ actual size.)

Ch, Calcium chloride tube. *KB*, Copper heater. *Gr*, Hollow mortar. *St*, Condenser tube. *LB*, Tube burner. *BB*, Movable burner. *T*, Wire gauze tunnel. *El*, Long roll of wire gauze. *Ek*, Short roll of wire gauze. *R*, Runnels of sheet iron. *AS*, Asbestos disc. *as*, Asbestos wrapping.

redness by the tube burner, as shown in the illustration, with flames of approximately equal height, which may be very accurately regulated.

The combustion tube rests on a combustion stand 250 mm. long. The four legs are of a height such that the tube lying in the V-shaped notches is the same height as the delivery tube of the three-way cock. In the upper edges of both long sides are two narrow parallel gutters *R*, which carry a coarse piece of iron gauze, bent 'to a U-shaped section, in the region of the filled portion of the tube. This gauze forms a tunnel-shaped space 180 mm. long which encloses the combustion tube. (Gauze tunnel *T*.)

The Electric Combustion Furnace. Of the different electric micro-combustion furnaces found in business, those of the firms of W. C. Heraeus, Hanau a. M,[1] and Kirchenbauer, Singen b. Pforzheim,[2] have been used by the Kaiser-Wilhelm Institut in the last five years, for the determination of carbon and hydrogen. The tube burner has been proved very practical with both furnaces. The electric heating of the tube filling is preferable to heating by gas, as the experimenter is exposed to scarcely any 'heat from radiation, the air of the laboratory is not made worse [3] by the gases of combustion, and the temperature of the tube remains constant to $\pm$ 10° C. during a whole day's combustion. Particularly in continuous work, when heating with gas, it is almost unavoidable that the temperature of the room by the evening should be higher than that of the balance room. According to private communications, long electric heaters have also been very much approved in other laboratories. Electric combustion tubes, however, are supplied with a movable electric burner and a mortar also heated electrically.

The movable electric burner can serve its purpose excellently in works in which substances with the same or very similar properties are always being analysed. In scientific laboratories, on the other hand, combustion cannot be according to a schedule, for many substances must be burnt according to their type in order to obtain correct results. It is therefore preferable to use the movable gas burner, as by this means the heating of the substance can be undertaken with constant observation of the decomposition and combustion, and the burner, and therefore the temperature,[4] can be regulated accordingly.

The electric heating mortar is not usually suitable for Pregl's

[1] B. Flaschenträger : *Z. angew. Chem.*, **41,** 840 (1928). *Mikrochem.*, **2,** 15 (1931).
[2] W. Füner : *Mikrochem.*, **10,** 66 (1932).
[3] The formation of steam can influence the carbon value. *Cf.* F. Hernler : *Mikrochem.*, Pregl's Festschrift, p. 148, 1929.
[4] The regulation of temperature by electric heaters is impracticable during the short time of the combustion.

proportions, and a variation in the filling of the tube [1] is therefore required for it.

Even to-day, to my knowledge, it is not possible to make a really satisfactory constant temperature electric heating mortar. Since the same amount of current always passes through the heating unit, a rise in temperature occurs from morning till night which exceeds the permissible amount. With such an apparatus, leaving current impulse out of consideration, the desired temperature would only be retained if the heat supplied as electric energy were equal to that given off from the insulation, which, in practice, is not attainable. Also constant temperature cannot be obtained by moving the regulating resistance.[2]

The solution of this technically somewhat difficult question appears possible, if a contact thermometer or a bimetallic rod fixed in a relay is attached. As the manufacturers of such apparatus do not take demands of this kind into account, it is best to heat with a long electric heater, but to heat the substance and the mortar with gas.

The Absorption Tubing

The air-tight connection of the calcium chloride tube between the neck of the combustion tube on the one hand and the soda-lime tube on the other, requires special consideration, because it was gradually found by many tedious experiments and failures that indiarubber, although an absolutely indispensable material for the organic micro-analyst, possesses many properties which render it unsuitable for the purpose. Firstly, it is hygroscopic ; secondly, it is very often porous originally or becomes so in use through production of capillary cracks or injuries ; and thirdly, it is permeable to carbon dioxide.

Its hygroscopic properties were very evident from the blank tests ; if these were carried out without any precautions, the increase of weight of the calcium chloride tube was found to be about 0·1 mgm., even when the potash apparatus showed no increase. This increase in weight of the calcium chloride tube did not occur when the rubber connection was previously carefully dried *in vacuo*, or, still better, if it had been in position for some time on the hot neck of the tube whilst this was pre-heated. This led to the conclusion that the hygroscopic character of the rubber was the cause of the increase. Further experiments, however, showed that it was not advisable to dry the tubing in this energetic manner

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[1] B. Flaschenträger's heating mortar is manufactured for the filling with lead peroxide which F. Pregl had given up again in the meantime.

[2] Dekaline kept at boiling point by electric heating is not safe from the risk of fire. If Dekaline from the vertical tube comes in contact with the heating winding of the long heater in front of the mortar it also burns.

before carrying out an analysis, as in that case hydrogen values were obtained which were rather low, particularly in compounds with high hydrogen content. This fact is easily explained by absorption of water, during prolonged contact, by the previously dried rubber, which is not completely removed, during the short period of the analysis, by the current of hot air.

These experiments led finally to an impregnation of the rubber connections, which reduced these drawbacks of the rubber colloid to such an extent that, even without a Mariotte flask—*i.e.*, without any regulation of the pressure—it is often possible to obtain accurate results.

In quantitative experiments on the hygroscopic properties of rubber tubing, M. Boetius has recognised the humidity of the air, especially on sultry summer days when the relative humidity is great, as the cause of the increase in weight of the calcium chloride tube. M. Boetius therefore recommends that the treated rubber tubing, whilst not in use, should be kept in a desiccator over phosphorus pentoxide. The moisture absorption drops to a minimum when the freshly treated tubing is wrapped round with a spiral band of tinfoil, 6–7 mm. wide and not too thin, so that the separate windings overlap. The tinfoil coating is fixed by means of strongly sized paper.

The Impregnation. Thick-walled absorption tubing of about 8 mm. outer diameter and 2–2·5 mm. bore is used. A narrower bore is unsuitable, because the considerable expansion of the tubing, when passed over the side tubes, affects its soundness just as much as the pinching and pressure necessary to put it on the tubes. The inner surface of the tubing must be perfectly smooth. For connecting the neck of the combustion tube with the calcium chloride tube, lengths of 20 mm. are used, and for connecting the latter with the soda-lime tube, lengths of 25 mm. ; these are placed in a small flask with pure molten paraffin wax and evacuated with the water pump on the boiling water-bath. As soon as the contents have ceased to foam, air is readmitted in order to force the molten paraffin into the finest interstices of the rubber. The evacuation and readmission of air are repeated until at the highest attainable vacuum no further bubbles arise on the tubing. This impregnation process requires half to one hour. The pieces of tubing are then allowed to drain while still warm, wiped outside, and the paraffin inside removed with cotton-wool wound round a steel wire. When in use the tubes are often wiped inside with a small wad of cotton-wool, moistened with a very small amount of glycerine, which is wound round a wire. Subsequently the tubing is wiped through with a dry wad of cotton-wool free from loose fibres in order to remove any excess of glycerine.

At present the firm of P. Haack, Vienna, provides tubing for this purpose, the quality and dimensions of which are excellent. Before use the paraffin wax must be removed with cotton-wool and a little benzene. This tubing is always used by the Kaiser-Wilhelm-Institut. No trouble is ever caused by it.

The Heating Mortar

Lead peroxide has the property of obstinately retaining a definite amount of water, even at rather high temperatures. In order to obtain correct hydrogen values, it is therefore necessary to maintain the lead peroxide at a constant temperature not only during the combustion, but also when preheating the tube. This is done with a heating mortar. The first mortars consisted of solid cylindrical copper mantels. They were heated to 180° with a micro-burner; the temperature was measured by a thermometer let into the top. These mortars are to be recommended even to-day, on account of their simplicity, where a constant gas pressure may be relied on.

In order, however, to ensure a constant temperature even with varying gas pressure, hollow mortars with a boiling liquid, e.g., cymene (b.p. = 176° C.) or Dekaline (b.p. = 188°-190° C.) is now generally used. A. Verdino's heating mortar [1] is seen in Fig. 7 in conjunction with the gas-heated tube burner; it has been in use almost daily for four years and has been found excellent in every respect. Leakage

FIG. 11.—Heating mortar.

can be removed at once through the tightening of a screw and the resin deposited after use for some time is easily removed by pyridine bases and benzene.

Recently H. Lieb [2] has recommended a very modern glass heating mortar according to Schöbel by the firm of Schott and Gen., Jena, after testing for two years in the Medizinisch-Chemischen Institut of Gratz University. This heating mortar (Fig. 2), of transparent Duroglass, is in shape and measurement copied fairly accurately from F. Pregl's hollow metal mortar. The hollow cylinder, which is 70 mm. long and 35 mm. external diameter, surrounds a central chamber to hold the combustion tube. The
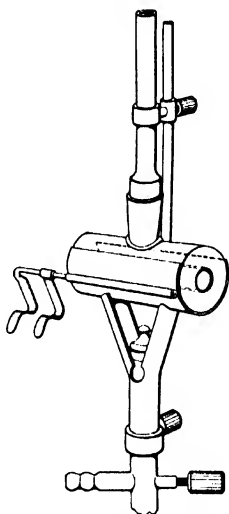
[1] A. Verdino : *Mikrochem.*, **9**, 123 (1931) ; by A. Orthofer, Instituts-mechaniker des Medizinisch-Chemischen Instituts des Universität Graz.
[2] H. Lieb : *Mikrochem.*, **14**, 263 (1934).

central chamber is of 14 mm. bore except where the neck of the combustion tube passes through ; here, the bore is 6 mm.   The top of the mortar contains a standard ground-in joint (2), in which the upright tube, 350 mm. long, is placed.

The hollow space holds about 40 c.c. of hot liquid and, for use, is about half filled with Dekaline. The mortar fits in a semi-cylindrical metal groove, which is lined inside with asbestos paper. The' mortar is clamped, by the inserted vertical tube, to a glass rod fixed vertically in the groove.   The copper heater is fixed in a metal tube soldered on to the groove.   The liquid is heated by a gas micro-burner built in the support. The metal groove may have a round opening over the flame, since Duroglass can be heated directly.   To avoid delay in boiling, small pieces of pumice stone at boiling point are sometimes brought into the heating liquid.

The glass mortar has the advantage over a metal one in that the boiling and the amount of heating liquid can be watched, and cleaning is easily accomplished.   According to the experience of the author the risk of breaking is very small.

The Absorption Apparatus

Second to the combustion tube, the absorption tubes are the most essential part of the whole apparatus. Since the weights of water and carbon dioxide formed in an analysis are very small in proportion to that of the absorption apparatus, it is essential to a really successful determination that the absorption apparatus should be filled with the greatest care. The exact dimensions of the apparatus shall first be described, because these, even by the
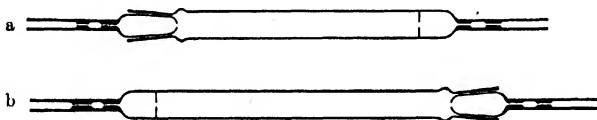
FIG. 12.—Absorption tubes of the firm of P. Haack, Vienna. a, Calcium chloride tube.   b, Soda-lime tube.   (⅓ actual size.)

best glass firms, are seldom checked with the necessary care. Trouble is caused to the beginner, particularly, through apparatus not according to specification, if he relies on the material delivered, and especially if he neglects to check the bore of the capillaries. Anyone experienced in glass-blowing can modify the capillaries to the correct dimensions.   Immediately on delivery, I myself have usually checked the capillaries of the absorption apparatus with platinum or silver wire, the breadth of which is readily determined

with a micrometer screw-gauge to $\pm$ 0·02 mm., and can strongly recommend this to all professional colleagues.[1]

The calcium chloride tube (Fig. 12, a) consists of a cylindrical thin-walled glass tube about 90 mm. long and 8-9 mm. external diameter. The tube is constricted on one side and carries on the other a ground-in stopper 12-14 mm. long. The water and gases enter the ante-chamber of the apparatus at the position of the constriction[2] through the fused on tube. The double capillaries with the ante-chamber cause a variable diffusion potential, which ensures a high constancy in weight of the apparatus. The ante-chamber is separated from the absorption part of the apparatus by a thin diaphragm. The diaphragm possesses a small central perforation, of diameter 0·3-0·5 mm., for letting moisture and gases through it. If the perforation is too small, then it is easily obstructed by water in the combustion of substances very rich in hydrogen, which brings the whole system to a standstill, because the Mariotte flask cannot overcome the surface tension of the water. In such cases the thin membrane of water must be vaporised by warming the diaphragm, or the pressure of the system raised by pushing back the movable burner about 30-50 mm.

The connecting tube of the calcium chloride tube has an external diameter of 3·3-3·5 mm., equal to that of the neck of the combustion tube, and is 25-30 mm. long. It is twice constricted to a capillary. For the capillaries which, according to the diagram in the third edition of this book, are about 2 mm. long, F. Pregl prescribes a diameter of 0·1 to at most 0·2 mm. The apparatus of the firm of P. Haack illustrated in Fig. 12 is twice narrowed to capillaries 5 mm. long. The gases and moisture streaming through have therefore about double the friction to overcome, and, especially if the diameter is less than the lower limit of 0·1 mm., must be very strongly aspirated through with the Mariotte flask.

Recently I have tested absorption apparatus with capillaries 5 mm. long and 0·2-0·25 mm. broad, for constancy of weight and found them to correspond with requirements, even in hot summer days of relatively high humidity. In the combustion of substances very rich in hydrogen there is often considerable trouble in driving the condensed water through capillaries of 0·1 mm. diameter into the ante-chamber by means of a hot forceps and the time for weighing out for the next determination may be lost, so that the 0·2-0·25 mm. broad capillaries are preferable.

On the other side of the calcium chloride tube, a ground-in

[1] This type of checking should really be learnt by glass firms, and all apparatus checked by it before sending out.

[2] Even the first potash apparatus of F. Pregl was supplied with ante-chambers at the side.

hollow stopper 10–12 mm. long forms a second ante-chamber. The hollow space of this stopper communicates on the one side with the inner space of the apparatus through an aperture 0·2–0·25 mm. wide and on the other side passes into a connecting tube similar to the preceding one.

The **soda-lime tube** (Fig. 12, b) is exactly like the calcium chloride tube, except that the filling chamber is 100 mm. long in order that a larger number of analyses may be possible without re-filling.
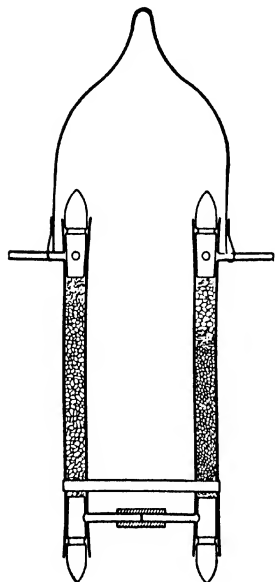
FIG. 13.—B. Flaschenträger's absorption apparatus with rotatory stoppers.

Finally the absorption apparatus constructed by F. Blumer [1] with two revolving ground-in stoppers should be discussed. It consists of a thin-walled glass tube for the absorption agent, which by turning the stopper can be placed in open communication with the unconstricted connecting tube, or closed as required. This ensures complete closing of the filling against the outside air and, consequently, very constant weight. The two-way cock is, however, difficult to wipe; indeed, the weight of the apparatus is usually only reproducible to ± 0·02 or even ± 0·05 mgm. This is caused principally by the necessary stopper-grease.

B. Flaschenträger [2] has been successful in doing away with several of the disadvantages of the Blumer absorption apparatus, so that constancy of weight is ensured. Fig. 13 shows the shape and size of these new tubes. In order to prevent stopper-grease from working-out, the spindle of the stopper is lengthened and carries a ring-shaped groove, 2 mm. broad and 0·5 mm. deep, 5 mm. from the bore. The lower part only of the stopcock is greased with lanoline (adeps lanæ). The groove prevents the creeping-up of the grease, so that the upper part (5 mm.) remains completely free from it. The apparatus is weighed full of oxygen, therefore the entire combustion can be carried out in this gas. The tubes are not very easily wiped; it is therefore easy to loosen or pull out the stopper in the process. If the tubes are once more opened after releasing the excess pressure of oxygen, they lose about 0·1 mgm. weight. The greasing of the

[1] *Ber. dtsch. chem. Ges.*, **50**, 1712 (1917), quoted by J. V. Dubsky.
[2] B. Flaschenträger : *Z. angew. Chem.*, **39**, 717 (1926).

stopcock requires more care than cementing with glass cement. The stoppers of the small tubes are smeared by means of a micro-spatula and then screwed home. Before removing the small tubes, after the combustion, the stoppers are turned through 180°. After wiping and cooling, and just before introducing into the balance, the stopcocks are opened for a short time, during which operation they can safely be held by the top and connecting tubes. For the analysis, both of the absorption tubes are held together by a rubber ring, which spans both tubes with a slight tension. They are hung up on a small stand by a wire stirrup and protected by asbestos shields from the heat radiated by the mortar.

Finally, the Friedrich [1] absorption apparatus, which can be closed, may be noted. This has been developed from F. Pregl's practical, rod-shaped construction. It is closed on both sides by means of similar glass stoppers with connecting tubes. Analogous to Blumer's apparatus, as improved by Flaschenträger, a transverse groove is placed in each glass stopper, which is only greased below this. The small connecting tubes pass into a gas channel, which opens towards the outside by a bore-hole in the stoppers. There is a small bulge on the ground joint of the stopper; if the stopper is turned so that the opening of the gas channel and the bore-hole are in the bulge, the apparatus is open. It is closed by a small turn of the stopper. Stainless steel clamps and springs attached within the apparatus prevent the stopper from falling out. Like Flaschenträger's apparatus, this is filled with oxygen before weighing.

The Mariotte Flask

This serves to maintain a definite and easily adjustable reduced pressure in the absorption train, to overcome the friction in the same to such an extent that approximately atmospheric pressure is maintained at the rubber connection between the combustion tube and the calcium chloride tube. As indicated in the diagram (Fig. 7), it consists of a levigating bottle of 1–2 l. capacity, into the lower tubulature of which a glass tube about 4 mm. thick and 2 mm. aperture is fixed, by means of a perforated cork stopper, in such a manner that it can be rotated to form a one-armed syphon. The glass tube is bent at right angles at both ends. Both outer angles are perpendicular to one another. The long portion should reach at least to the upper opening of the bottle. Rubber stoppers are
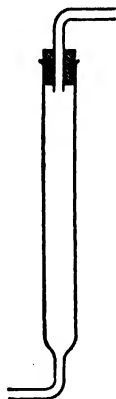
Fig. 14.—Calcium chloride tube with two connecting tubes bent at right angles. (Actual size.)

[1] A. Friedrich : *Mikrochem.*, **10**, 329 (1932).

unsuitable for the connection of this syphon with the Mariotte flask because they hold the glass tube too stiffly, whilst, when inserted in a cork stopper, any position is readily maintained. The upper tubulus of the levigating flask is provided with a doubly bored rubber stopper, one hole of which is closed with a glass rod or glass stopcock which should be removed or opened when the apparatus is not in use, in order to prevent water being drawn out in spite of the vertical position of the syphon, if the temperature rises. Through the second perforation in the stopper, a glass tube of 3-4 mm. bore, bent twice at right angles, is inserted, as shown in the diagram, in such a position that it reaches to the bottom of the bottle.

The free end of the glass tube, which is directed downwards, is connected with a small calcium chloride tube (Fig. 14) by means

FIG. 15.

of ordinary rubber tubing. The calcium chloride tube and the soda-lime tube are connected during analysis by means of absorption tubing, 30–40 mm. long. It is best to stand the Mariotte flask on a tripod with supports at the sides (Fig. 7).
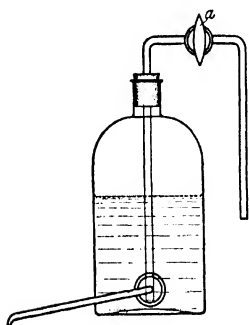
In Fig. 15, J. Unterzaucher's stopcock, *a*, is shown. About this very simple and practical innovation, Dr. Unterzaucher kindly wrote to me :

" The replacing of the syphon of the Mariotte flask in a vertical position at the beginning of every carbon and hydrogen determination, in order to avoid leakage during weighing, can be cut out in a very simple way, which saves time and trouble.

" By bringing a stopcock, *a*, into the inlet tube of the Mariotte flask, the closing of which prevents suction of air and the accompanying leakage of water when the syphon is in the lowered position, it is possible to leave the position of the syphon unchanged while the filling of the absorption apparatus lasts.[1]

" Accordingly, it is only necessary to close the stopcock directly before taking off the absorption tubes at the end of an analysis, in order that, on continuation of the work after connection of the tubes mentioned, the original conditions of temperature and pressure may be restored by simply reopening.

" For many years, we have found this device to be best, and it has also been taken up and approved by other laboratories."

[1] In combustions of substances very rich in hydrogen, after calcium chloride filling has been in use for some time it may be found that alterations in resistance occur through the added water. A slight alteration of the height of the syphon at once remedies this.

Reagents [1]

Sodium Hydroxide. A 5 per cent. solution is used for filling the pressure regulator. For one filling, 40 gm. in 800 c.c. of water are taken.

Potassium Hydroxide. For filling the bubble-counter, the 50 per cent. potassium hydroxide described on p. 76, with which the azotometer also is filled, is used.

Asbestos. Commercial Gooch crucible asbestos is used for the asbestos wads. It is purified as follows: The coarse fibres and the parts as fine as powder are first mechanically separated from the medium-sized ones, and finally washed in a coarse suction filter with distilled water. It is then transferred to a large platinum or glass dish and digested for five hours with concentrated hydrochloric acid. The asbestos is washed with hot distilled water, in the filter already used, until the filtrate shows no traces of chloride (see halogen test, p. 100). Most of the water is removed from the purified asbestos by slowly drawing alcohol through. The asbestos is placed in a crystallising dish, loosened with a forceps, and left to dry for several hours in a desiccator over phosphorus pentoxide. The last traces of moisture are removed in the drying-oven at 120° C. and the pure white asbestos may now be kept in a wide-necked bottle.

Asbestos purified in this manner neither retains water nor temporarily absorbs carbon dioxide [2]; it is not to be regarded in any way as a source of error.

Silver Wire or *Silver Wool.* These can be obtained absolutely pure from A. Bühne and Co., Freiburg in Breisgau, and from P. Haack, Vienna. It is advisable to reduce the silver wire before use, in a current of hydrogen in a glass tube, and afterwards to ignite it in a stream of oxygen. The same procedure is adopted for the silver fillings of used tubes which are charged with halogen and sulphur, in order to regenerate them for further use.

The mixture of *Lead Chromate* and *Copper Oxide* is made by impregnating wire-form copper oxide with an equal weight of lead chromate. The commercial lead chromate may be used directly after powdering finely in a porcelain mortar.[3] The copper oxide is crushed in the mortar into smaller pieces, 4–5 mm. long, and used for the oxidising mixture without further preliminary treatment.

The mixture of lead chromate with copper oxide is prepared in a nickel-steel dish on a strong blowpipe flame. Fifty grams of powdered lead chromate are strewn on to 50 gm. of red-hot copper

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[1] Unless otherwise stated, all reagents are obtainable from E. Merck, Darmstadt.

[2] *Cf.* M. Boetius: "On the Sources of Error in F. Pregl's Microanalytical Method for the Determination of Carbon and Hydrogen," p. 44.

[3] F. Pregl previously prepared the lead chromate by precipitating a dilute solution of lead acetate with potassium dichromate.

oxide, stirring very thoroughly with an iron rod so that the whole surface of the copper oxide is coated with the molten lead chromate. If the copper oxide is properly treated with the chromate, then, on the one hand, the somewhat porous copper oxide is covered with a compact layer of lead chromate, and, on the other hand, the alkali which always adheres in small amounts to copper oxide is converted into chromate. The value of this oxidising mixture has been fully confirmed by J. Lindner [1] and M. Boetius,[1] after it had already obtained the fullest recognition from many authors.[2] Only H. Hennig [3] replaces the oxidising mixture described by one of copper oxide pumice and lead chromate pumice, because copper oxide by blowing with the reducing flame may absorb considerable amounts of carbon, which is only given up again by degrees.

Lead peroxide is the most critical reagent and has therefore been repeatedly the object of exhaustive researches.[2] In the case of substances which consist only of carbon, hydrogen and oxygen, only an oxidising tube filling is needed. If, however, nitrogen is a constituent of the molecule, only two agents are available for fixing oxides of nitrogen, namely, metallic copper and lead peroxide. Of these, copper must be excluded, because it is very readily oxidised in the continuous presence of excess of oxygen. M. Boetius [4] has been able to show in very interesting experiments that by conducting air over metallic copper alteration in the density of the air occurs in consequence of the loss of oxygen, which causes, in the blank test, a decrease of about 0·05 mgm. in the weight of the absorption apparatus. Lead peroxide, therefore remains, with its disagreeable property of taking up moisture temporarily and absorbing carbon dioxide in the dry state ; the latter is ultimately given up in presence of small amounts of water ; the peroxide is however the only means by which substances of any composition can be reliably analysed. F. Pregl and, later, J. Lindner [5] have already recognised that lead peroxide is not detrimental to correct values for hydrogen if it is heated to temperatures of 180°–200° C. In this range of temperature, the absorbed water is quickly given up again on the one hand, and, on the other, the stage of drying reached is not

[1] J. Lindner : *Ber. dtsch. chem. Ges.,* **59,** 2806 (1926), and M. Boetius : " On the Sources of Error in Pregl's Method of Microanalytical Determination of Carbon and Hydrogen."

[2] F. Kopfer : *Z. analyt. Chem.,* **17,** 28 (1878) ; H. Weil : *Ber. dtsch. chem. Ges.,* **43,** 149 (1910) ; M. Dennstedt : *Z. analyt. Chem.,* **42,** 417 (1903) ; J. Lindner : *Ber. dtsch. chem. Ges.,* **59,** 2561 (1926) ; M. Boetius : " On the Sources of Error in Pregl's Method of Microanalytical Determination of Carbon and Hydrogen."

[3] H. Hennig : Reprint from the reports of the Math.-physik. Kl. Sächs. Akad. Wiss., Leipzig, **85,** 182 (1933).

[4] M. Boetius : " On the Sources of Error in the Microanalytical Determination of Carbon and Hydrogen," p. 51.

[5] J. Lindner : *Ber. dtsch. chem. Ges.,* **59,** 2806 (1926).

so high that absorption of carbon dioxide can occur. In order that the foregoing conditions for the use of lead peroxide should be accurately maintained, Pregl's hollow mortar should be used, which gives a constant value for the vapour pressure of the water-lead peroxide system. On these grounds, it is absolutely necessary either to prepare a totally irreproachable lead peroxide oneself or to test the suitability of a commercial preparation most thoroughly.

Purification of Lead Peroxide.[1] One hundred and fifty grams of commercial lead peroxide are digested, with frequent stirring, in an evaporating dish with concentrated nitric acid (density 1·4), for two hours on the water-bath. After standing for one to two hours it is repeatedly washed by decantation with distilled water whilst stirring until nitric acid can no longer be detected in the wash-water by diphenylamine-sulphuric acid.

The slimy residue is then almost completely dried on the water-bath and cut into small cubes of about 2 mm. edge with a spatula. The pieces are rotated in a roomy wide-necked bottle, either by hand or by a slowly rotating lathe. They thus polish one another and finally the smooth pieces are sieved. After remoistening and drying, the powdered portion can again be cut up into cubes and treated as before. The black [2] preparation is put in a wide-necked bottle. After six hours' ignition in the combustion tube, excellent hydrogen values can be obtained with it.

The description of the lead peroxide used earlier is omitted, because it has the drawback of being too quickly exhausted in combustions of substances containing nitrogen, and a further drawback is its swelling on heating.

Krönig Cement. This is prepared by melting 1 part of white wax with 4 parts of colophony and is poured into cylindrical moulds.

Dekaline (b.p. 188° C.) or *cymene* (b.p. 176° C.). The former is usually preferred. Commercial Dekaline (Deutsche Hydrierwerke, Rodleben bei Rosslau) can be used in the mortar much longer if it is shaken two or three times with concentrated sulphuric acid, washed with water, dried carefully, and finally distilled. The first light oil, which contains small amounts of acid, is removed, and the middle fraction (b.p. 186°–190° C.) is collected directly in a suitable stock bottle. This purified Dekaline is much less inclined to resinify than the commercial product.

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[1] In the supplement to the 3rd edition it is stated that the firm E. Merck, Darmstadt, no longer manufacture Pregl's granulated lead peroxide. I have used lead peroxide made by Schering-Kahlbaum, Berlin, for three years with the fullest satisfaction.

[2] Impure preparations are not black, but brownish to reddish-brown.

Calcium Chloride. Pregl's fused, groat size for microanalysis is used. The preparation which has been made for a long time by E. Merck shows a loss of 1·2 per cent. on ignition. By using soda-lime simultaneously as absorption agent for carbon dioxide good results for hydrogen were always obtained.

Anhydrone (magnesium perchlorate) obtainable from I. T. Baker, Chemical Co., Philippsburg, New Jersey, U.S.A., can be put into the absorption apparatus without further treatment.

Soda-lime (p.a. finely granulated, E. Merck). In order to obtain a satisfactory value for carbon dioxide, soda-lime must not be too dry nor too moist.[1] No quantitative specification can be given at present as to the correct moisture content of the soda-lime for the filling, and it therefore happens occasionally that the beginner in particular must find for himself the right degree of moisture for the preparation used, after some wrong analyses in the first place.

Every new delivery of soda-lime is ignited in order to obtain the moisture content necessary for correct analyses. For this, an ignited porcelain crucible of about 8 c.c. content is 'placed in a weighing bottle and weighed with it on a macro-balance. Then 4–5 gm. of soda-lime are weighed in, and the crucible ignited on a Bunsen flame at dark red heat for ten minutes and cooled in a desiccator over phosphorus pentoxide. The crucible is re-weighed in the closed weighing bottle and the loss on ignition determined. Absolutely reliable values for carbon are obtained if the commercial soda-lime is moistened with as much water as will add 33 parts of water to 100 parts of ignited anhydrous soda-lime.

To prepare a larger amount of soda-lime, a wide porcelain dish, containing 50 gm. of Merck's soda-lime, and a crystallising dish containing 7·00 c.c. of water are placed in a large desiccator. After about two to three days the soda-lime will have absorbed all the water. If the desiccator were evacuated quickly to about 5 mm. Hg and left closed, the water would be absorbed in half the time. If moistened soda-lime is lacking, one places a weighed amount (for one to two fillings) on a filter-paper and sprays, with rather more than the correct amount of water because the filter paper absorbs some, from a 10 c.c. measuring cylinder. Then the soda-lime is rolled about on the filter-paper to moisten it uniformly and brought at once into the absorption tube. In the literature, the moistening of soda-lime is usually explained by the fact that dry soda-lime does not absorb carbon dioxide as well as moist. I can, however, personally confirm, from qualitative experi-

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[1] *Cf.* p. 63.

ments, the statement by A. Friedrich, that the moistening of the
soda-lime only serves the purpose of preventing the removal of the
last trace of moisture from the gases passing over it. By the use of
phosphorus pentoxide instead of calcium chloride, and of dry [1]
soda-lime, the weight of the soda-lime tube remains constant in a
blank test, and in analysis the carbon dioxide is quantitatively
absorbed. If, on the other hand, the dry soda-lime is used in
combination with calcium chloride, the soda-lime tube increases in
weight in a blank test and too high a value for carbon is obtained
in analysis. This further shows that the soda-lime in the U-tube
must not be moistened.

The A. H. Thomas Company, Laboratory Apparatus and
Reagents, Philadelphia, West Washington Square, U.S.A., has sold
an excellent absorption agent for carbon dioxide for some years
under the name of Ascarite. Soda-lime tubes filled with this
material absorb on the average about 500 mgm. of carbon dioxide,
that is, about three to four times as much as soda-lime. The
progress of the absorption is indicated by a marked coloration of the
absorbing agent, so that it is always possible to judge whether a
given filling will still suffice for further analyses. The exhausted
filling is not so easily removed from the tube as soda-lime, as
absorption is always accompanied by consolidation and an
increase of volume, for which reason the filling can only be removed
without danger after soaking over night in slightly acidified
water. The mechanical removal of the exhausted filling with
a soft wire, as in the case of soda-lime, is almost impossible with
this reagent.

A further circumstance, which must receive special attention,
is the fact that in contrast with soda-lime the vapour-tension of the
water in Ascarite is extremely small. It is consequently necessary
to remove the last traces of water from the calcium chloride used
for filling the U-tube of the bubble-counter, the calcium chloride
tube, and the Ascarite tube, by heating over an illuminating flame
in a round-bottomed flask evacuated by a water pump. Otherwise
the water-vapour from the calcium chloride tube would be absorbed
by the Ascarite in the soda-lime tube and would thus cause an
erroneously high result in the carbon determination.

The firm of E. Merck, Darmstadt, supplies a very similar and
equally efficient preparation under the name of "Natronasbest" [2]
(soda-asbestos).

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[1] As obtained from E. Merck (19 per cent. moisture) and not ignited.
[2] According to a private communication from Dr. E. Wiedemann, Chemische
Fabrik, formerly Landoz in Basle, with this preparation, simultaneously using highly
dried calcium chloride, very good analytical results are obtained.

Setting up a New Apparatus

The apparatus for the determination of carbon and hydrogen can be set up on any laboratory bench, on which all parts of the apparatus can be closely examined by daylight or electric light. Near the right side of the table there should be about 40–50 cm. of free surface for setting-up the two steel bottles, in order that the gases may not have to travel too far to the pressure regulator. In case of want of room these bottles may be placed on a stand under the working table.

After cleaning with chromic acid, water, and distilled water, the pressure regulators are about three-quarters filled with 5 per cent. caustic soda solution (p. 18) ; the bell jars are put in and connected with the gasholders by means of artificially matured rubber tubing, which passes through two precision pinchcocks (Fig. 7). If the gases are brought directly from the steel bottles, the pinchcocks are superfluous and the connection with the valves is made with two carefully cleaned glass tubes, which touch the tubes of the pressure regulators and are connected with the valves by rubber tubing, of a length such that the bell-tubes of the pressure regulators, which are normally immersed to a depth of 7 cm., can be raised about 2 cm. without stretching the tubing. For the further passage of the gases the tubing is connected from the delivery tubes of the pressure regulators to the three-way cock, and this stopcock is greased with a little vaseline so that it readily slips into the ground-in joint.

The electric combustion furnace must next be closely tested and standardised at a temperature of 550° C. with the rheostat which belongs to it. Above all, by the measurement of the temperature with a thermo-element, proof should be obtained that the heating casing of the furnace is brought uniformly to glowing heat.[1] For this, the rod end of a thermo-element is placed exactly in the middle of the furnace and the temperature standardised at 550° C. $\pm$ 20° by means of the rheostat. The corresponding position of the slide is carefully marked. Afterwards, the initial heating is conducted with the resistance cut out, and the slider is first placed on the mark when the heater begins to glow. Finally, the temperature is checked about 2 cm. from both ends of the heating wrapping (heating rods). Further heating of the furnace is unnecessary, if on looking at the heater a good red glow can be detected in its whole length.

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[1] In distances of 1 to 2 cm. from the lateral chamotte insulation the temperature is 30° to 50° C. lower than that inside of the heater. This difference cannot be prevented, for purely technical reasons, and does not affect the results.

Filling the U-tube

The U-tube is cleaned with chromic acid, water, and alcohol, and dried by means of the pump or in a drying-oven. It is filled by first inserting a small wad of cotton-wool into the side tube of the sealed limb and then filling in granulated anhydrous calcium chloride through the ground-in joint of the U-tube, until about two-thirds of the tube is filled. A small wad of cotton-wool holds this filling in position, and the empty portion of the U-tube up to the level of the side-tube leading to the bubble-counter is then filled with unmoistened soda-lime. A wad of cotton-wool is added in such a fashion that the soda-lime cannot fall into the bubble-counter. The glass stopper is carefully warmed and screwed into the ground-in joint with Krönig's cement. By means of a finely tapered glass tube, there is now introduced, drop by drop, into the bubble-counter enough 50 per cent. caustic potash solution to ensure that the tapered end of the gas delivery tubes is immersed about 2 mm. in the caustic potash. Should more than this be added, the excess is removed by blowing into the other end. After cleaning with a small wad of cotton-wool, the U-tube is provided with a wire stirrup and suspended by it from the hook of a stand.

Filling the Combustion Tube

First the measurements of the tube are checked exactly to those given on p. 22, and it is washed with hot chromic acid, distilled water, and alcohol. It is best dried by drawing air through with the water-pump under gentle heating. To make a choking plug of 6–7 mm. long, a small portion of purified asbestos (p. 33) is ignited for about ten minutes on a platinum lid of about 4–5 cm. diameter, with the non-luminous flame of a Bunsen burner.

The choking plug serves to produce the greatest gaseous friction of the whole system at the end of the tube. On its compactness depends the height of the effective column of water, which is required in the pressure regulators in order that the gases may pass through the system at a rate of 4 c.c. per minute (measured by the Mariotte flask). One must be careful, therefore, so that no incompletely burnt substance may arrive in the absorption apparatus on account of sudden expansion of gas.

With the platinum-tipped forceps, two to three small wads of ignited asbestos are introduced into the tube and pushed up to the position of attachment of the neck with a glass rod of about 5 mm. diameter with sharp edges ; it is pressed against the wall of the tube and packed carefully together. It is now advisable to test

the degree of gaseous friction, that is, of the density of the plug. For this purpose, the combustion tube is attached to the bubble-counter and it is determined whether, at a pressure of 40–60 mm. of water in the pressure regulator, about 3–4 c.c. of gas pass through the tube per minute.[1] It should also be considered that a somewhat larger friction is set up against the gases through the subsequent filling of the tube ; this, however, is easily compensated by a head of 10–20 mm. in the bell tube.

Lead peroxide is then poured on to the choking plug to a height of 20–25 mm. and distributed by careful tapping. Before further filling, the lead peroxide adhering to the wall of the tube must be removed by means of a plug of cotton-wool attached to a wire. To avoid contamination, by lead peroxide dust, of the silver which now follows, a loosely packed asbestos layer 2 mm. thick is placed between them. The adjoining layer of silver is about 40 mm. long in a gas furnace ; its length depends on the mortars. For a distance of 10 mm. between the mortar and the tube burner, 5–10 mm. of the silver filling should be in the wire gauze roll of the burner. With electric heating, the mortar is brought nearer, preferably up to 5 mm. from the furnace, on account of the already cooler chamotte lining, and the silver wool is inserted up to the beginning of the heating coil. A layer of asbestos, 2 mm. thick, is now pushed up to the reduced silver, the tube filled up with the mixture of lead chromate and copper oxide, to a length of about 140 mm., in the case of a gas furnace, and finally a stopper of silver wool about 15–20 mm. long keeps the filling in place.

The heater for electric furnaces is furnished with wheels, so that the long heater may be moved against the movable burner. By this, one can also heat the colder parts of the tube between the chamotte lining after the combustion, and remove residual substances. As previously stated, the combination of the long electric heater and movable gas burner is preferred to-day. With this, the good conductivity of silver can be utilised by bringing a 30 mm. silver layer up to the layer of copper oxide and lead chromate, the length of the latter being chosen so that the former projects 5 mm. beyond the portion of the tube heated by the long heater. If, towards the end of the combustion, the movable burner is moved up to the long heater, then heating the end of the silver layer for a minute suffices to ensure that the last fragments of the substance near the long heater are burnt. By this method, in the case of electric heating the moving of the long heater becomes superfluous. Around the filled part of the tube a short roll of iron wire gauze, 50 mm. long, is first placed and then a long one, about 11 mm. in diameter, which does

[1] *Cf.* p. 47, Calibration of the tube with the bubble-counter.

not fit too closely (Fig. 10).[1] To fix the tube in the mortar, it is wrapped in a strip of asbestos paper, over the layer of silver adjoining the lead peroxide. This strip also prevents the production of an air current through the mortar, which would cause temperature changes.

According to A. Verdino,[2] as much Dekaline is brought into the mortar as will reach a height of 10–20 mm. in the ascending tube on boiling. The mortar should be separated from the neighbouring wall of the combustion stand by not more than 1 cm.

The mouth of the tube is closed with a well-fitting cork with fine pores, and the side tube is connected with the U-tube by means of matured rubber tubing.

A perfectly filled tube, if carefully treated, can be used for at least 200 analyses, if it is not unduly heated and if the layer of lead peroxide is never exposed to a higher temperature than 200° C. If substances containing halogens or sulphur are burnt consecutively, the silver wool adjacent to the boat is considerably affected. It is then better to remove this after about ten to twenty analyses and to replace it by new silver wool.

The method of filling the tube described, which may at first sight appear somewhat complicated, is the result of much consideration and a whole series of experiments : the lead peroxide ensures the retention of the higher oxides of nitrogen probably formed from nitro-, nitroso-, and hydroxyazo-compounds. If the organic material also contains halogen and sulphur, lead peroxide, which is recommended and used for these, is not in all cases sufficient ; particularly if it already contains lead nitrate. The best reagent for the absorption of halogen is heated metallic silver, and lead chromate is suitable for retaining the oxides of sulphur. By placing these two reagents in front of the lead peroxide, the burden on it is removed and its only function, that of absorbing the higher oxides of nitrogen, remains unimpaired. The possibility of removing the part of the filling which is most affected, after some time, keeps the remaining filling of the tube permanently serviceable.

Filling the Absorption Tube

In order to avoid difficulties later, new apparatus is first tested accurately, as on p. 28, for the correct breadth of the capillaries, and afterwards placed [3] for an hour in warm dilute hydrochloric acid, then washed, by suction, with water, distilled water, and alcohol and dried in the oven at 110° C.

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[1] In electric furnaces the tube is used without long rolls of iron wire gauze.

[2] A. Verdino : *Mikrochem.,* **9,** 123 (1931).

[3] After wiping new apparatus a loss in weight is observed, which is due to the loss of material from the surface. A. Friedrich, *Z. angew. Chem.,* **36,** 481 (1931), recommends placing the apparatus for some time in hot dilute hydrochloric acid.

The Calcium Chloride Tube. The filling of the absorption tube with calcium chloride or anhydrone (magnesium perchlorate): To prevent scattering of dust, a small loose pad of cotton-wool is placed on the diaphragm of the ante-chamber. On this are put either two or three pieces of coarse, porous calcium chloride or else, if only the groat size is at hand, two small capillary tubes, 10–15 mm. long, 1·5 to 2 mm. bore, and a length of about 20 mm. of groat-sized calcium chloride.• On this is placed a small pad of cotton-wool, 2–3 mm. thick, and groat-sized calcium chloride added up to the ground-in joint. The calcium chloride is covered with a large pad of cotton-wool and the joint cleaned with cotton-wool twisted round a coarse steel wire. The ground joint and stopper are now warmed gently, in a just non-luminous small flame, some Krönig glass cement is placed on the stopper, and the still warm stopper is screwed into the ground joint without streakiness. The excess of cement is removed after cooling, first mechanically, and then by a rag moistened with benzene. Since calcium chloride absorbs and fixes small amounts of carbon dioxide, the carbon values produced, without preliminary treatment, by the first analyses would be too low. The apparatus is therefore attached, by the end opposite to the joint, to a Kipp apparatus for the development of carbon dioxide. The air is first displaced by carbon dioxide, in the same direction as in later analyses, then the open end is closed and the apparatus left for ten minutes with carbon dioxide under pressure. Afterwards the carbon dioxide is removed by sucking 100 c.c. of air through by means of the Mariotte flask.

If anhydrone is used as absorption agent, the saturation with carbon dioxide may be omitted. In continuous work, anhydrone filling has the advantage of being able to absorb about 200 mgm. of water, whilst calcium chloride begins to cake [1] even after absorbing 80–100 mgm., and must be renewed.

The Soda-lime Tube. This is filled in the same way as the calcium chloride tube ; a plug of cotton-wool about 5–6 mm. high is brought through the mouth and on this a layer of 30 mm. of groat-sized calcium chloride or anhydrone. A layer of cotton-wool, 2–3 mm. thick, forms the boundary between the calcium chloride and the soda-lime which follows, and serves the purpose of preventing the calcium chloride from rolling out when the soda-lime is renewed. The soda-lime, moistened as on p. 36, is now filled up to the ground-in joint, covered with a cotton-wool pad, and the stopper cemented in and cleaned. Through this tube also, 100 c.c. of air are aspirated.

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[1] Phosphorus pentoxide is not approved as absorption agent, according to personal communications from my professional colleagues. *Cf.* also H. Hennig, p. 34, footnote 3.

One filling can safely absorb 120–140 mgm. of carbon dioxide. When ready for the analysis, the absorption tubes are closed by caps of rubber tubing and kept near the balance on a metal stand, which supports them at two points only. Finally the calcium chloride tube (Fig. 14) connected with the Mariotte flask is filled. The filling used is renewed after some months.

• It is interesting to note that tubes filled in this way with calcium chloride weigh about 6 gm., with soda-lime about 7 gm., and with Ascarite about 9 gm.

Cleaning and Wiping the Absorption Apparatus

Before conducting the first determination of carbon and hydrogen it is most advisable to practice the cleaning and wiping of the apparatus several times and thus to check the constancy of weight.

For cleaning the connecting tube, a steel wire, of about $\frac{3}{4}$ mm. diameter and with roughened ends, is used. On this as much cotton-wool as will exactly fit in the connecting tubes is wound with the fingers. For cleaning and wiping the external surfaces, two flannel cloths, about 6 cm. × 10 cm., and four chamois leathers of the same size are used. They
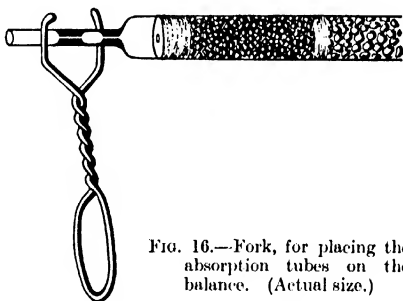
Fig. 16.—Fork, for placing the absorption tubes on the balance. (Actual size.)

are kept in three roomy glass jars and when not in use are protected from dust by inverting the jars. The leathers must always be perfectly clean; they must be thoroughly washed with lukewarm soapy water containing a few drops of ammonia and then dried on a string at room temperature. Before use, the flannel cloths are moistened. They are first placed in distilled water, then wrung out, rolled in a clean, dry towel, and most of the water removed by squeezing hard. The moist cloths are then placed in a glass box and left in it with the leathers for an hour. On very humid summer days this precaution may be omitted.

The caps of the apparatus are first removed and the tubes placed on a soft support. The calcium chloride tube is held in a moist cloth, and the connecting tubes cleaned as far as the constrictions by turning the above described wire in them. After seeing that no particles of rubber are left in them, the external surfaces are cleaned, holding a flannel cloth in each hand and wiping the tube twice from the middle over the connecting tubes to the ends,

rotating the apparatus. It is then wiped similarly with the first pair of chamois leathers, and then with the second, and replaced on the metal stand.

The soda-lime tube is cleaned in the same way. After wiping, the apparatus must not be handled. Five minutes after removal from the stand or from the neck of the combustion tube, the calcium chloride tube is placed on the hook of the left balance suspension by means of an aluminium fork (Fig. 16), and the counterpoise is placed on the right pan. The final weighing is made five minutes after bringing into the balances, e.g., ten minutes after the removal. The soda-lime tube is similarly treated. Cleaning and weighing of both tubes requires exactly fifteen minutes. With expert handling the weight of the absorption apparatus after repetition of this should be reproducible with an accuracy of $\pm$ 0·005 mg.

The absolute necessity of retaining these weighing times, which Pregl had fixed through numerous systematic tests, can easily be seen.

Every piece of apparatus undergoes an apparent diminution in weight after handling and wiping, which is principally due to the warming of its surface. The consequence is that, immediately after wiping, an increase of weight is observed, which is considerable during the first five minutes, namely, 0·1 mgm. or even a multiple of this. Between the fifth and tenth minute it is only a few hundredths of a milligram, and after fifteen minutes a value is reached which remains unchanged after a further half-hour, if all the necessary conditions are fulfilled in the weighing room and for the apparatus.

Very occasionally it has been observed that the apparatus shows a maximum weight after wiping and then becomes steadily lighter. An investigation conducted by F. Pregl in association with K. Brunner has incidentally led to the knowledge that this is due to the excessive dryness of the chamois leather used for wiping the apparatus, which causes electrostatic charges on the glass surface of the apparatus. It can be completely avoided by keeping the chamois leathers in a glass bottle into which the moist flannel cloths are sometimes introduced for an hour.

According to Pregl, the absorption apparatus can only be weighed with the necessary accuracy if the balance room has a temperature equal to, or higher than, that of the combustion room. If the tube is brought into a very cold balance room, the contraction of the air inside the tube draws in a weighable quantity of water vapour, and positive errors up to 0·07 mgm. and more are found.[1]

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[1] *Cf.* M. Boetius : "On the Sources of Error in the Microanalytical Determination of Carbon and Hydrogen by F. Pregl's Method," p 91.

To guard against this in the case of the soda-lime tube, F. Pregl recommended placing a doubly folded piece of moistened flannel over it during the combustion. Increases in weight through moisture absorption were not observed with electrically heated combustions, therefore I gave up the cooling of the soda-lime tube some years ago. Without moistened flannels, results are obtained accurate enough to satisfy me and also foreign colleagues.

Pressure Relations in the Apparatus, and Use of the Mariotte Flask

Considering the pressure relations in the whole system, the gas pressure, corresponding to the head, in the pressure regulator will be approximately constant from that point to the choking plug. In consequence of the gaseous friction caused by the plug, this allows only equal amounts of gas to pass in equal times, and also gives rise to a sudden decrease of pressure behind it, which, if no absorption train were connected, would fall to atmospheric pressure in the neck of the combustion tube.

· If we now connect the absorption apparatus, without the Mariotte flask, the pressure is increased in consequence of the capillary constrictions and the gas friction, till a value is reached which corresponds with the friction of the whole system up to the last capillaries of the soda-lime tube. It is therefore easily seen under what pressure the whole apparatus would come, and what demands would be made on the air-tightness of the rubber tubing, if the current velocity of 4 c.c. per minute can only be reached in this way. In order to avoid this excessive pressure, a constant reduced pressure of the required size is produced at the connection of the Mariotte flask to the end of the soda-lime tube, so that atmospheric pressure exists at the most critical position of the apparatus, between the choking wad and the first capillary constriction of the calcium chloride tube.

These considerations led to the use of the Mariotte flask, which F. Pregl rightly named a safety precaution.

Use of the Mariotte Flask

A large number of blank tests has shown, that in spite of the use of unexceptionable oxygen and matured tubing for introducing the gases, increase in weight occurs in both absorption tubes if the pressure in them is greatly reduced. On the other hand, it was found that the figures obtained in the combustion of easily combustible substances were lower than those required by theory, if the pressure in the absorption train, and particularly in the connection

between the neck of the combustion tube and the calcium chloride tube, was higher than that of the atmosphere. This suggested that carbon dioxide and water were aspirated through the warm rubber connection in the former case and expelled in the latter.

Various experiments were carried out in connection with this matter, the results of which are sufficiently interesting to be quoted here. Carbon dioxide contained in a glass burette over mercury was connected with rubber tubing, the end of which was closed by a glass rod. After a few minutes the reduction in volume could be observed. If the experiment lasts sufficiently long, the gas vanishes from the enclosed space until only a small amount is left in spite of the production of a reduction of pressure which often amounts to more than 200 mm. of mercury. This phenomenon takes place to a slightly less extent and in a longer time, when tubing is used which has been treated with crude molten vaseline *in vacuo* in the manner already described. If, on the other hand, air free from carbon dioxide is enclosed over mercury in a gas burette, with a piece of tubing which has been exposed for a considerable time to carbon dioxide under pressure, and through which air has been 'drawn for a few seconds only in order to remove carbon dioxide, at any rate from its bore, one observes an alteration of the mercury level of 1-2 mm. only, even after several hours. It follows that the carbon dioxide which is absorbed by rubber is liberated extremely quickly by passing air through it, that is, the migration of the carbon dioxide in the rubber colloid in the direction from a higher to a lower partial pressure is very rapid.

These experiments show that the selective absorption of carbon dioxide by rubber, and its capacity for liberating it again in the direction of lower concentration, is, at most, merely reduced by impregnation with vaseline, whereas its ordinary capacity to absorb and liberate water vapour is almost entirely removed by this process ; for tubing treated in this manner, when tested by a blank experiment, transmits at most 0·02-0·04 mgm. of water to the calcium chloride tube in spite of being heated by the hot neck of the combustion tube.

From these experiments it follows also that any carbon dioxide which penetrates into the body of the rubber connecting tube during the short period of the combustion is almost quantitatively removed by the following stream of air free from carbon dioxide. From this standpoint pressure equalisation by the Mariotte flask appears to be entirely unnecessary. It is otherwise, however, with the faultiness of the gas-tight connection which occurs after frequent use, even with impregnated rubber tubing, and often occurs suddenly and unnoticeably. In such cases, obviously, in accordance with the

results of blank experiments and analyses, a positive pressure inside must lead to a loss of carbon dioxide, while if the pressure within is below that of the atmosphere, moist air will be absorbed.

In this case we are dealing, therefore, with losses or increases caused by leakage of the rubber connections, whether through capillary cracks which have been produced in the course of time or through definite porosity of the tubing. In either case the tubing between the neck and the calcium chloride tube is more exposed to injury than that between the two absorption tubes on account of the greater degree of heating.

The use of the Mariotte flask is not absolutely essential for the success of the analyses if the rubber connections are faultless. In spite of this it is strongly recommended ; it serves :

1. For the calibration of the tube with the bubble-counter (p. 47).

2. For slow aspiration of air through the freshly filled absorption tubes (p. 42).

3. For testing for leakages of the apparatus (p. 59).

4. For constant checking of the current speed during the combustion and for easily measuring the 100 c.c. of air required for the quantitative replacement of gas.

Finally the Mariotte flask enables us to absorb the products of combustion accurately without erroneous increase or loss in weight even if the rubber connections are slightly faulty.

The Calibration of the Tube with the Bubble-counter

For the quantitative conversion of organic substances into carbon dioxide and water a certain time of contact of the gases produced with the red-hot tube filling is necessary, *i.e.*, the speed of the gas current must not exceed a maximum value. In numerous analyses of substances combustible with difficulty it was found that with a gas speed of 4 c.c. per minute the combustion is absolutely complete.

In order to overcome the gaseous friction of a correctly filled tube, with the stated gas speed which is measured by the Mariotte flask, a pressure of 50–80 mm. head of water is used.

The speed of the current is shown by the bubble-counter. We use it as a permanent check for the regulated speed of the gas in the red-hot combustion tube as well as to standardise the correct aspiration of the Mariotte flask after the insertion of the absorption apparatus.

The standardisation of the pressure regulators for a gas speed of 4 c.c. per minute is carried out on the tube, after igniting for six hours, as follows :

The pressure regulator is first set with a water-head of 50–70 mm. and the bubbles passing through the bubble-counter in ten seconds

are determined, using a stop-watch. Then the calcium chloride tube of the Mariotte flask is connected with the neck of the combustion tube by means of rubber tubing and the syphon of the Mariotte flask adjusted so that the bubble-counter again shows the bubble frequency previously determined. The gas passed through the tube in five minutes is measured by the water which flows away into a measuring cylinder placed under the syphon. The calculation of the speed of the current from the figures obtained may be illustrated by an example :

The first adjustment of the pressure regulator gave 18 c.c. of water in five minutes for nine bubbles in ten seconds ; thus the speed of the current did not amount to 4 c.c. as required, but only to 3·6 c.c. per minute. The correct number of bubbles, which is adjusted by raising the head of water in the bell gasholder, is calculated thus :

$$\text{Required count} = \frac{\text{volume required}}{\text{volume found}} \times \text{bubble count of the test.}$$

In our case : $\dfrac{20 \times 9}{18} = 10$ bubbles in 10 seconds.

This speed of 4 c.c. per minute is checked, and then the second pressure regulator is brought to the same frequency. This adjustment is maintained thereafter for the tube unless it has to be readjusted owing to warping of the tube or displacement of the lead peroxide.

It should be noted that an excess of suction of 5–10 mm. by means of the Mariotte flask is less objectionable than a positive pressure in the absorption train, which may lead to losses of carbon dioxide and water, whereas, with properly impregnated rubber tubing, neither water nor organic vapours are evolved under increased suction.

The result of all these considerations is an apparatus in which, on theoretical grounds, a number of conditions has been fulfilled in a manner much more complete than has so far been the case in ordinary macroanalyses. It causes the gases to issue from the combustion tube at a perfectly definite velocity which is easily established, and which is determined empirically, and thus ensures that each gas particle remains in contact with the admittedly suitable tube filling (universal filling) for a definite period of time. This is attained by the choking plug placed near the exit of the gases from the combustion tube. Its effect differs essentially from that of a pinchcock which regulates the entrance of the gases to a combustion tube in which there is no gaseous friction, for this latter arrangement, at best, only ensures regularity of the gas stream, but not a

uniform time of contact with the red-hot tube contents. As the stated effects of the choking plug are only possible when the pressure remains constant, and as this is difficult to maintain with certainty by means of a pinchcock, it was achieved by means of a small glass gasholder, the pressure regulator, which enables one always to control the desired gas velocity by means of the necessary pressure and makes an excess pressure quite impossible.

The Course of the Determination

Weighing

Solids are weighed in boats of platinum,[1] porcelain,[2] or resistance-glass.[3] It is advisable to weigh substances containing arsenic or mercury, as well as compounds of alkalies and alkaline earths which are mixed with potassium dichromate, in boats of porcelain or resistance-glass [4]; all other substances can be analysed successfully in platinum boats. The boats are boiled with dilute nitric acid (1 : 1) before each analysis, ignited on a platinum hook or by means of a platinum-tipped forceps, in a non-luminous flame, and then placed on the copper block of the small desiccator.

Because of the heat-conductivity of the copper block, the platinum boat cools to room temperature in a few seconds and the weighing may be begun.[5] The boat is removed from the desiccator, which stands near the balance, on to the left-hand balance pan by means of a platinum-tipped forceps, the aluminium counterpoise is placed on the right-hand pan and the boat weighed accurately to 0·001 mgm. The boat is then transferred to the analysis notebook and filled with 3–5 mgm. of the substance to be analysed, with the help of a microspatula. Larger quantities are unnecessary for the combustion unless the carbon and hydrogen contents of the substance are very low. For organic salts of phosphoric acid, salts of gold, platinum, alkaline earths and other salts, from which the weights of carbon dioxide and water are very low, it is advisable to increase the weight to 5–7 mgm. In many cases less than 3 mgm. should be used. With irreproachable apparatus, good results can be obtained with even as little as 2 mgm. Analyses with amounts of 1 mgm. are rare and should only be valued in conjunction with an analysis on a normal weight.

As the weighing of the absorption train before and after the determination is only accurate to $\pm$ 0·01 mgm., the error of the

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[1] W. C. Heraeus : Hanau a.M.
[2] Berlin porcelain.
[3] P. Haack, Vienna.
[4] Old platinum boats may also be used.
[5] About ten minutes must be allowed for boats of porcelain and resistance-glass.

soda-lime tube for a weight of 3 mgm. of a substance with 70 per cent. carbon is limited to a variation of 0·08 per cent. carbon, but for a weight of 1 mgm. of the substance the possible variation lies outside Pregl's limit of $\pm$ 0·2 per cent. Although, for this weight, many analytical results have been obtained within this limiting error, yet one has to reckon with an inaccuracy of at least $\pm$ 0·4 per cent.

Before replacing the boat on the balance it is held with the forceps and brushed on the sides and the bottom with a dry, dust-free sable-hair brush, to remove particles sticking to the outside. The weighing is made with the same accuracy as the previous one, the boat is then placed on the copper block of the small desiccator and the cover put on.

Extremely volatile solids, such as cyclo-octanone, camphor, tricyclene can neither be weighed into boats nor brought into
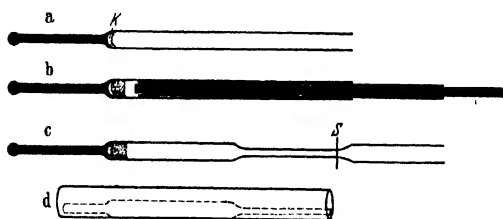
Fig. 17.—Preparation of the capillary for weighing solid, extremely volatile substances. (Actual size.)

(*a*) Capillary for weighing, with solid handle, in which a crystal of potassium chlorate (*K*) is fused. (*b*) The substance is pushed from a capillary tube into the capillary for weighing by means of a glass rod. (*c*) Drawing out the capillary and sealing at *S* after weighing. (*d*) Platinum cylinder, in which the capillary is pushed into the combustion tube.

capillaries in liquid form. Such substances are pushed into a capillary closed at one end, which is afterwards sealed, as in Rast's micro-determination of molecular weight (p. 242).

For this a thin-walled capillary of about 2–2·5 mm. diameter is prepared from a test-tube in a non-luminous Bunsen flame, sealed in the flame of the micro-burner about 40 mm. from the mouth, and drawn out to a solid handle about 15 mm. long (Fig. 17 (*a*)). A crystal of potassium chlorate is introduced into the sealed end of the capillary, and fixed there by careful melting.[1] The capillary is then placed on the copper block of the small desiccator and weighed accurately to 0·001 mgm. after a few minutes. Three to four milligrams of the substance to be analysed is removed from a watch-glass by means of a second, longer capillary tube open at both ends, which exactly fits into the bore of the weighed capillary. The

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[1] This precaution is not absolutely essential.

substance is taken up by pressing in with a spatula, and the residue adhering to the outside is carefully brushed off with a marten hair brush. The weighing capillary is placed, with the handle underneath, in a short glass tube, 25–30 mm. high, which is sealed at the bottom and stands vertically in a block of wood or cork stopper. The capillary containing the substance is now inserted up to 5–10 mm. from the bottom (b) and the substance is pushed into the weighing capillary by means of a thin glass rod ; the glass rod is first removed and then the capillary. The capillary is softened in the flame of the micro-burner about 20 mm. above the substance ; it is drawn out to a finer capillary in the outer part of the flame and weighed after cooling (c). It is advisable that the weighing capillary should not be drawn out narrower than 0·5 mm., because otherwise it may be blocked by recrystallisation of substances of high melting-point, whereupon the capillary bursts on further heating and glass splinters fall into the combustion tube.

The weighed capillary is sealed as below and brought to the apparatus. In a cylinder of platinum foil, 4–5 mm. long and closed at one end, the capillary is placed in the combustion tube after breaking the handle in the middle and breaking the sealed tip (d). On heating the closed end of the cylinder, the whole of the platinum foil becomes so hot that the fused substance can no longer be retained in the capillary itself and emerges into the tip. It is therefore not advisable to allow the tip to project beyond the cylinder, especially also as then, during the course of the combustion, the tip would break off over the flame and fuse into the combustion tube. The combustion of liquids by F. Pregl's method, described later, is still carried out like this in platinum cylinders at the Kaiser-Wilhelm-Institut. Likewise, for combustions with mixtures with potassium dichromate the tube is protected by surrounding the boat with platinum foil.

Viscous substances (oils) with low vapour pressure are brought into a boat with a very thin glass rod, taking care that the outside of the boat is not wetted.

For weighing **liquids with low vapour pressure,** capillaries made from test-tubes are used, which are subsequently sealed. Capillaries about 100 mm. long and 1 mm. bore are melted in the middle, as in the diagram (Fig. 18), to a drop ; this is drawn out to a glass rod about 25 mm. long (b) and sealed through in the middle (c). The ends of both handles are rounded off to small spheres. A crystal of potassium chlorate is then placed at the sealed end of such a capillary, and is there fixed by melting (d). About 20 mm. from the closed end, the capillary is softened, drawn out to a hair-capillary in the outer flame, and broken off after 25–30 mm. (e). It is best in

4—2

this case also, not to draw out the capillary to less than 0·1 mm.[1]
If decomposition of the potassium chlorate occurs whilst still liquid
in the fine capillary tube, the
tube bursts all too readily.

After weighing the capillary
accurately to three decimal
places, it is held in a forceps
and air driven out by carefully
warming, without melting the
potassium chlorate. The still
warm tip is inserted into the
liquid, which is drawn in on
cooling gradually. The liquid
which has entered (3–5 mgm.)
may be jerked on to the potas-
sium chlorate at the bottom
by sharp taps, holding the
capillary point upwards be-
tween the thumb and fore-
finger, but a hand centrifuge
is better. Traces of the liquid
still remain on the inner walls
of the fine point, which would be
lost on opening the hair capil-
lary. To remove these, the
empty portions of the capillary
are drawn quickly through the
flame twice or three times, the
fine end (*f*) is thus sealed, and
the tube is weighed after a few
minutes. The capillary tube,
deprived of part of the handle
and the tip, is best brought into
the combustion tube in the
cylinder of platinum foil (p. 51).

Fig. 18.—Preparation of the capillary
for weighing liquids. (Actual size.)

(a) Production of a glass drop in the
middle. (b) Drawing out the same.
(c) One half produced by sealing off in the
middle. (d) After melting the introduced
crystal of potassium chlorate on to the
floor of the capillary. (e) After drawing
out the open end to a capillary (first
weighing). (f) After introduction of the
liquid and sealing. (g) The capillary after
removing the handle and breaking off the
point, lying on platinum foil at the
moment of introduction into the combus-
tion tube.

Liquids with large vapour pressure, such as ether, are weighed
by J. Pirsch's method,[2] e.g., in a capillary 1 mm. in diameter, which
passes into a very fine hair-capillary, from 20–30 mm. long at a
distance of about 30 mm. from the handle. The capillary, prepared
and weighed as on p. 51, is dipped at the point into a small deep

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[1] This should still be seen with the naked eye.
[2] J. Pirsch: *Ber. dtsch. chem. Ges*, **65**, 865 (1932). The method of weighing
published for the determination of molecular weights has also proved very good for
weighing very volatile liquids for carbon, hydrogen, and nitrogen determinations,
and is preferable to the method of F. Pregl and G. Petridis.

dish containing the substance to be analysed; about 6 mm. of the wider portion of the capillary project above the dish. The capillary is filled by raising with a heated forceps for a few seconds and re-immersing afterwards in the liquid. On cooling, the liquid rises in the capillary. If about 3–4 c.mm. are found in the widened part, the capillary is removed with a bone forceps; thereby air presses the liquid back. The extremely fine capillary prevents evaporation of the substance. After half an hour on the balance, no loss on weight is to be observed. Because the capillary is brought from the balance into the combustion tube unsealed, all warming must be avoided.

Quantitative Drying of Non-hygroscopic Substances

According to the adhering solvent and the melting-point of the substances, drying is carried out at different temperatures and pressures. Substances which decompose readily must be dried at the lowest possible temperature and the adhering solvent is best removed in a high vacuum.

For drying at ordinary temperatures the boat containing the weighed substance is best placed in a desiccator, which contains the appropriate drying agent; this is evacuated either by the water pump or by the high vacuum. After weighing, the determination of the zero point of the balance must not be forgotten. The substance is of constant weight if after drying for at least an hour the weight is unaltered to $\pm$ 0·002 mgm.

If the substance should be dried at higher temperatures and under reduced pressure the usual pistols or F. Pregl's micro-desiccator may be quoted here, with which drying is carried out on the regeneration block.

The **micro-desiccator** (Fig. 19) consists of a glass tube 240 mm. long and of 10 mm. external diameter, which is narrowed to a hair-capillary in the centre. In one half, several layers of firmly compressed cotton-wool are followed by granulated calcium chloride to a length of about 50 mm., and this is held in place by a further layer of cotton-wool. The opening is closed with a rubber stopper, through which a thermometer capillary
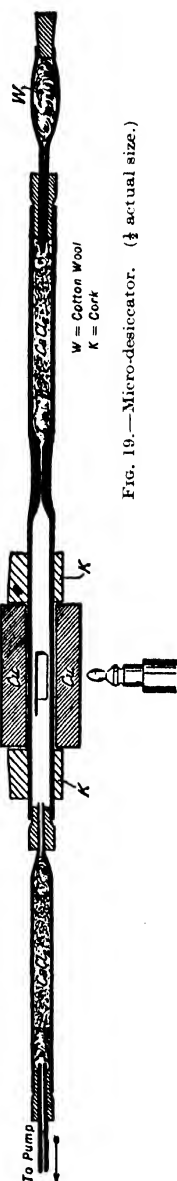
W = Cotton Wool
K = Cork

FIG. 19.—Micro-desiccator. (½ actual size.)

passes. This expands into an olive-shaped bulb, which is filled with compressed cotton-wool. The empty half of the main tube serves for the reception of the boat. The open end is also closed with a rubber stopper, through which is inserted the neck of a small tube filled with calcium chloride which is connected with a pump by an intermediate part. If one now evacuates it, the pressure in the micro-desiccator drops to the minimum attainable by the water-pump used, assuming that the capillaries are sufficiently fine. They are only intended to permit a minimal but constant movement in the drying chamber by the entry of extremely small amounts of dried air. The substance in the micro-desiccator is heated by insertion of the latter into the copper block (regenerating block) which is also used for drying the halogen tubes and is described in connection with these (Fig. 33). In order to prevent rotation around its axis, and therefore the upsetting of the boat, the half containing the substance to be dried is held between two corks which fit exactly, which prevent rotation by being pressed tightly against the copper block. Flat surfaces are also filed on both corks, which enable the micro-desiccator with boat and the contained substance to be laid on the bench top without rolling. After closing the rubber tubing leading to the pump with a screw clip, the pump is turned off. After a few minutes, pressure equilibrium is completely restored and one can transfer the warm micro-desiccator to the balance. The calcium chloride tube with its rubber tubing is then removed from the opening ; the platinum boat is withdrawn with a platinum hook, removed with the forceps, transferred to the copper block of the small desiccator, and weighed after two minutes.

Besides the micro-desiccator, which can be brought to any temperature whatever by direct heating in the copper block, a drying pistol [1] is also very useful. Its temperature can be accurately fixed over rather large intervals by the use of hot liquids of various boiling-points (alcohol 78° C., water 100° C., xylene 139° C., Dekaline 188° C.), and makes it possible to dry ten boats simultaneously in an aluminium groove 10–12 mm. wide, 7–8 mm. deep, and 250 mm. long. It also permits larger amounts of substances, which are used for a series of determinations, to be dried together.

Quantitative Drying of Hygroscopic Substances

In the case of hygroscopic substances, it is necessary to weigh both the empty and the filled boat in a weighing-bottle (Fig. 20). The handles are as thin as possible, to reduce the effect of warming it by handling to a minimum. The weighing-bottle should always

[1] W. Vetter, Heidelberg.

be kept in the balance case, except when carrying the boat to the drying pistol and back. Without special precautions, it should neither be brought into a desiccator nor heated to rather high temperatures, because the moisture deposit on the large surface must always be the same. If it is dried or enclosed warm, important differences in its weight appear, and one must wait a longer time before weighing, *i.e.*, until the weighing-bottle has assumed the temperature and humidity of the balance room. If the bottle has been held in the
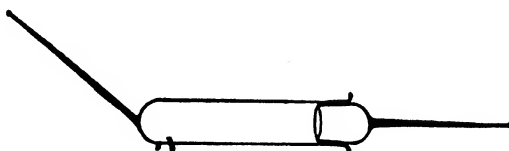
Fig. 20.—Weighing-bottle for hygroscopic substances. (Actual size.)

hand, it increases in weight on the balance ; it must be constant before weighing.

With extremely hygroscopic substances, absorption of water may occur and falsify results even whilst bringing the dried substance from the pistol into the weighing-bottle. The high-vacuum micro-desiccator has proved excellent for drying such substances for many years, in the microlaboratory of the Technical High School, Munich. Dr. J. Unterzaucher has been good enough to send me the following communication :

The High-vacuum Desiccator [1]

" The micro-analyst sometimes meets with considerable difficulty in the analyses of hygroscopic substances or those which are difficult to dry. This is due, on the one hand, to the refinements obtained through the micro-scale and, on the other, to the lack of suitable apparatus for meeting the difficulties hereby appearing.

" To obtain correct analytical results, the substance to be analysed must be freed completely from adhering solvent or moisture, *i.e.*, must be dried to constant weight. Pregl's [2] micro-desiccator suffices in many cases. It does not suffice in all, however, owing to the fact that the dried substance is brought again into the moist air during transfer from the micro-desiccator to the weighing-bottle and also because of the impossibility of using a high vacuum and maintaining it for any length of time. A. Stoll's and E. Wiedemann's [3] method, worked out for the micro-analysis of hygroscopic substances, describes a further development, but, owing to its low

[1] DRGM. The apparatus is made by the firm of W. Neumann, Glasbläserie, Munich, NW 2, Theresienstr, 78.
[2] F. Pregl : " Quantitative Organic Microanalysis," 2nd (English) Edn., pp. 68–70.
[3] A. Stoll and E. Wiedemann : *Helvet. chim. Acta,* 16, 200 (1933).

stability, the apparatus used has the disadvantage of being difficult
to manipulate.

" Frequent occupation with extremely hygroscopic substances
led the author to make an apparatus to which the drawback of the   ·
apparatus mentioned does not apply. The new high-vacuum
micro-desiccator is shown diagrammatically in Fig. 21, and exhibits
a well-known revolver desiccator (*a*). The substance is dried in
this, in a boat (*b*) in the weighing-bottle (*c*) under a high vacuum
and at a raised temperature. The weighing-bottle is square in cross-
section and slides in a device (*d*) inserted in the desiccator by means
of two attached vanes (*e*), so that the same remains with the boat
horizontal and cannot come in contact with the edges of the
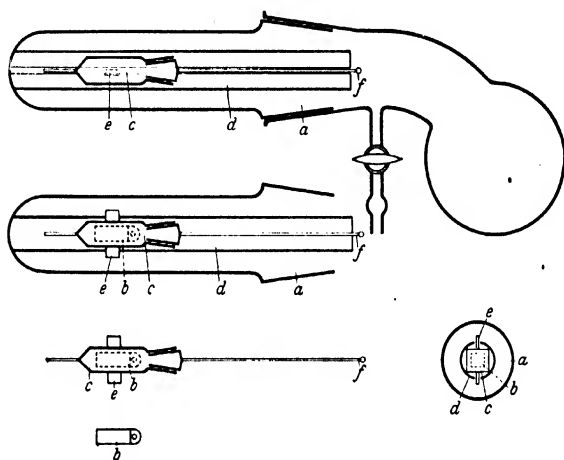
Fig. 21.—High-vacuum micro-desiccator.

desiccator on withdrawal. In order not to bring the substance into
the damp atmosphere after drying, the stopper of the weighing-bottle
is provided with a shaft (*f*) which projects beyond the sliding
device. This enables the weighing-bottle to be closed in the tube
of the desiccator. Suitable cavities for the vanes of the weighing-
bottle are placed in the sliding device, to prevent the bottle from
slipping or tilting the desiccator. The weighing-bottle and desiccator
are provided with screwed-in stoppers, made by the firm of W. K.
Heinz, Stützerbach.

" The apparatus is manipulated in the following simple manner :

" The closed weighing-bottle and the boat are always weighed
together, great care being taken that the final observation, especially
after withdrawing from the desiccator, should not be made until the
condition of the object to be weighed is completely adapted to that
in the balance case, which occurs after about fifteen minutes, giving

constant weight. After each weighing the zero of the balance is determined, in order to cut out errors resulting from a possible displacement of the same during the time of drying. By means of the long shaft of the stopper of the weighing-bottle, this is brought into the desiccator and pushed back until the vanes are placed in the cavities of the sliding device. Then one opens the weighing-bottle by turning the stopper and leaves the latter in the sliding device. After putting on the closing cap of the desiccator, supplied with fresh phosphorus pentoxide, the high vacuum is turned on and the part of the desiccator containing the substance is brought into the side bore of a drying-oven regulated according to the degree of drying required.

" The time of drying naturally depends on the nature of the substance. It has been found by experience that in extreme cases the attainment of constant weight requires several days and even weeks ; the latter particularly if drying must be carried out at lower temperatures.

" Following the drying and cooling, to abolish the vacuum air is allowed to flow in through a tube filled with phosphorus pentoxide or pumice and provided with a fine capillary at the other end. The desiccator is hereupon opened, the weighing-bottle closed, taken out of the desiccator, and brought to be weighed. The handling of the bottle is done either with chamois leather or with freshly-washed dry fingers.

" The advantage of the apparatus consists in the fact that it permits of the analysis of solids which are characterised by strongly hygroscopic properties and extremely stubborn retentivity of the solvent, to be dried for any length of time under a high vacuum and at a raised temperature, and to bring them to constant weight, avoiding contact with the moist air of the room. On account of its simplicity and great stability, the desiccator ensures most reliable and easy work.

" The high-vacuum micro-desiccator here described has proved excellent in our work and elsewhere for a whole series of substances with the above properties, the analyses of which by the previous apparatus was only partially successful, or was unsuccessful, therefore we use the former exclusively for all such work."

Preparation of the Combustion

It is considered best to adhere to the following systematic procedure, which is the result of experience gained in thousands of analyses, and in which the sequence of individual analyses is arranged in a correct and suitable manner.

The doors of the balance are first opened, in order to ensure " acclimatisation." The closing cap is then taken from the neck

of the combustion tube, the valve of the oxygen bomb or the stop-cock of the oxygen gasholder is turned on, and the three-way tap set for the passage of the oxygen. The mortar is heated with a large regulated flame in order to boil the heating liquid quickly and the tube filling is heated with the flame or with the electric heater of the long burner, the resistance of which is first completely cut out and is only inserted at the mark described on p. 38 when the heater begins to glow. The three-way stopcock is then closed by turning through 45 degrees, thus preventing the escape of the oxygen from the gasholder of the pressure regulator when the cork is removed from the combustion tube. The mouth of the tube is cleaned with a wad of cotton-wool wrapped tightly round a steel wire, the cork is again inserted, and the three-way stopcock brought into its original position. About 4 cm. from the position of attachment of the side tube ignition of the combustion tube is begun. The part heated is protected from overheating by the roll of wire gauze. Whilst the empty part of the tube is ignited for ten minutes, the flannel cloths are moistened as on p. 43, placed in a glass dish with the chamois leathers, and covered.

If Dekaline or cymene are boiled in the mortar, using a small flame, and if the electric furnace has attained the correct temperature by adjusting the slider on the mark of the resistance and the empty part of the tube begins to glow, then the valve of the bottle of compressed air or the stopcock of the air gasholder is opened. The bubble-counter, previously set for a current speed of 4 c.c. per minute, is now checked, both for air and oxygen. The valve of the compressed air bomb is closed, and the three-way stopcock opened for oxygen, so that the tube is filled with oxygen for the combustion whilst the weighing-out is done.

The Analysis

Every series of analyses is begun by weighing out a test substance (*e.g.*, alizarine, salicylic acid, cholesterol, etc.), as on p. 49. The weighed substance is brought on the copper block of the small desiccator to the apparatus. The absorption train is weighed exactly as described on p. 43.

The free time during the weighing is used for filling the Mariotte flask, and the absorption tubing is moistened with very little glycerine by means of cotton-wool wrapped round a steel wire.

The insertion of the weighed absorption tubes into the apparatus is carried out after first connecting both tubes together by means of the longer absorption tubing, with the glass portions in immediate contact. The shorter piece of rubber tubing is then drawn over the side-tube of the calcium chloride tube, to the middle of it, and the

connection with the neck of the combustion tube is similarly made with glass touching glass. Finally the calcium chloride tube, which is attached to the Mariotte flask by rubber tubing, is connected with the soda-lime tube. The syphon of the Mariotte flask must obviously be placed in the upright position. If the apparatus is completely gastight, no bubbles should rise in the bubble-counter. After this test for leakage, the current of oxygen is cut off from the bubble-counter, the cork removed from the mouth of the combustion tube, and the boat containing the substance is pushed into the combustion tube by lifting the copper block in the left hand in front of the mouth of the tube so that it touches the edge of the tube from below, then moving the boat absolutely horizontally completely into the tube by means of an ignited platinum-tipped forceps. By means of a clean glass rod with rounded edges it is carefully pushed up to about 40–50 mm. in front of the layer of silver wool filling. It is not advisable to push the boat nearer to the filling in the case of liquids and substances which melt at the temperature of the room, because, even with electric heating, the tube at 30–35 mm. from the ignited filling is about 5°–8° C. hotter than that further away. Hence, until the actual combustion begins, the substance might sublime back ; if only some of the substance arrives behind the heated zone, the analysis is spoilt. It is advisable to place substances of low melting-point at least 60–70 mm. from the tube filling. The tube is now closed with the cork and the short roll of wire gauze is placed so that its front edge just reaches to the handle of the boat.

Sometimes it is advisable to test for leakages. For this the syphon of the Mariotte flask is lowered to 30 degrees below the horizontal. If bubbles still rise in the Mariotte flask after a few seconds, the position of the leak can easily be found.

One then notes whether the bell gasholder is filled with oxygen and connects it with the apparatus by turning the three-way cock. The syphon of the Mariotte flask is now lowered until the bubble-counter shows the same bubble frequency as before the admission of the absorption train. The syphon remains in this position till the end of the analysis and should in no case be moved. After placing the copper heater[1] of the hollow mortar over the first capillary constriction of the calcium chloride tube the combustion may be started.

The Combustion

The short roll of wire gauze is heated with the full flame of the movable burner, which is just non-luminous. The heating of the tube is followed by a diminution of the bubble frequency in the

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[1] Copper heaters may also be prepared which warm both capillaries. These are only used in analysis of substances very rich in hydrogen.

bubble-counter ; after a short time this returns to the original frequency. After a few minutes most substances undergo alteration, especially if they are such as melt above 300° C. or are liable to decompose. They are characteristically melted, sublimed, distilled, or even carbonised. When these phenomena have ceased, the gauze roll is advanced some millimetres, and after some time, ten to twenty seconds, the burner is moved after it to about the same extent. Every time that the burner is moved forward the frequency of the bubbles is somewhat diminished ; it is therefore necessary to leave the burner stationary for so long, at least, that the original bubble frequency is restored ; therefore it is necessary to watch both the material to be analysed and the bubble-counter. If burnt without wire gauze, regulation of the strength of the flame is still at one's disposal.

It is necessary to be particularly careful not to advance the burner too quickly, because, by this, sudden formation of rather large amounts of gas may very easily occur, and the pressure set up is then transmitted against the direction of the gas current up to the bubble-counter. This leads to serious losses in most cases, because the vapour of the substance analysed passes behind the heated zone of the movable burner or even into the neighbourhood of the opening of the tube. Particular attention is paid to substances which form liquid drops in front of the hot long burner, which suddenly form gas if the movable burner is brought up too quickly. Substances which deposit charcoal and are very difficult to burn may be burnt without difficulty in platinum boats, if the burner is removed for a short time, after ignition for a longer time, and the cooled boat is again heated. The particles of charcoal then burn with the production of a shower of sparks.

Attention can only be given here to the precautions which must be most strictly observed by the beginner. From the first stages, the expert knows exactly how the combustion should best be regulated. Because he has to analyse the most varied substances in succession, he can, as a result of observations made in the course of time, burn every substance in the way necessary for it. About ten minutes suffice for the combustion with oxygen.

If the movable burner is moved up to the tube burner, the valve of the steel bottle of compressed air is opened and the three-way cock turned through 180 degrees, to admit air. A 100 c.c. measuring cylinder replaces the beaker under the syphon, and the air sent through the system is measured by the water collected in the cylinder. The tube is now heated to redness in less than ten minutes with the movable burner, starting about 70 mm. from the cork. During this time a boat for the next weighing is boiled and ignited, and one observes

that no condensation of water occurs on the capillary of the calcium chloride tube. For capillaries of the type prescribed, obstruction very seldom occurs on a weight of 4 mgm. of a substance containing up to about 9 per cent. of hydrogen. With substances very rich in hydrogen, the water is driven over into the ante-chamber with a nickel forceps heated in the flame of the movable burner. If one should have to leave the apparatus unwatched for some time, and if the stream of gas should come to a standstill through the water accumulated during this time, the capillary is at first warmed with the hot forceps. If the resistance is still not removed, the burner is pushed back a few cubic centimetres, but not so far that the alkali will enter the horizontal inlet tube of the bubble-counter. The water is then usually driven out of the capillary in a few seconds by the extra pressure. The same manipulation is used if the hole in the partition of the calcium chloride tube should be coated with water.

At the end of the second ignition, 40 c.c. of water have been collected in the measuring cylinder. The following fifteen minutes, corresponding with the further 60 c.c. of water, are used for weighing the next substance.

When 100 c.c. of air have been drawn through, the copper heater is removed from the calcium chloride tube, the syphon of the Mariotté flask is raised upright, and the rubber connection is removed from the end of the soda-lime tube, holding the tube in the right hand. The combustion tube is then disconnected at the neck, and the rubber tubing placed near the apparatus. Finally, the rubber tubing is removed from the calcium chloride and soda-lime tubes.

Cleaning and wiping of the absorption tubes is carried out as on p. 43, and the calcium chloride tube weighed first, ten minutes after removal from the apparatus. After placing the absorption tubes on the metal stand, the boat is removed from the tube with a platinum hook fixed into a glass rod ; it is ascertained with a lens if any ash remains behind to be weighed as residue ; the mouth of the tube is closed, the air supply shut off, and oxygen again led through the tube. After weighing, the absorption tubes are ready for the next determination.

From the beginning of the combustion to the final weighing of the soda-lime tube about fifty minutes elapse.

Calculation

The results of the analysis are calculated by logarithms.

Formula :

log (percentage of H) = log (wt. of H_2O) + log (factor H) + 2 − log (wt. of substances)
log (percentage of C) = log (wt. of CO_2) + log (factor C) + 2 − log (wt. of substances)

An example should make this clear ; the beginner should write this up in his notebook.

Alizarine.	Calcium Chloride Tube.	Soda-lime Tube.				
			97313 04884	91381 43573	Theory 3·36%H	69·98% C
3·318	49·00	20·25				
0·123	48·06	12·05	49553	49553	Found 3·29% H	69·99% C
3·195	0·94	8·20	51750	84507	Error — 0·07%	+ 0·01%

Blank Tests

These are necessary to ensure that the apparatus is working satisfactorily, and to detect any sources of error appearing during the course of the operation. The blank test is carried out in exactly the same way as a combustion, the only difference being that no substance is inserted in the tube.

On the assumption that the blank test is correctly carried out, sources of error which can arise from gases, rubber tubing, tube filling, or absorption agents are indicated, if increases in weight above 0·05 mgm. for the calcium chloride tube and above 0·02 mgm. for the soda-lime tube can be detected.

If we first consider only the errors in the apparatus up to the absorption tubes, then especially we have to consider unsatisfactory air and oxygen which contain organic vapours in their containers or absorb them on the way to the combustion tube. Correct manipulation of the gasholder was described in detail on p. 17. Impurities do not usually occur in gases from steel bottles, because, owing to the risk of explosion, the valve is sealed with water only. For the same reason, the needle-valve must not be greased or oiled.[1] If, nevertheless, faulty increases in weight through the gases should be suspected, then one must decide which of the two gases is responsible by means of a blank test, using air only. If this test yields no increase in weight, then oxygen is led through three or four times, when the absorption tubes must show a correspondingly larger increase in weight. The gases can also be taken up by the adsorbed organic constituents of rubber tubing on passing through this. To check this source of error with certainty, tubing of different lengths is tested. In most cases trouble through unsatisfactory tubing is removed after steaming out for one hour. All errors due to organic vapour lead to increase in weight of the absorption tubes, especially the soda-lime tube. If gases which are not open to objection cannot be obtained, a catalyser tube (Fig. 6) is introduced in front of the bubble-counter.

In the further part of the apparatus (excluding the U-tube,

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[1] The manometer of A. Hofer's valve is inscribed : " Keep free from oil, on account of the risk of explosion ! "

which has been discussed in connection with the absorption train) up to the combustion tube, there are practically no sources of error if glass is in contact with glass throughout.

Errors from the filling of the combustion tube are usually due entirely to the lead peroxide. It is found occasionally that a freshly filled tube gives up small amounts of water after ignition for six hours. The same thing may be observed if the tube has not been used for some time, when the calcium chloride tube increases in weight and the soda-lime tube is constant. Remedy: Longer ignition.

Increases in the weight of both absorption tubes, particularly the soda-lime tube, are due to lead peroxide which has not been prepared according to the instructions and gradually gives up traces of nitric acid, due to its manufacture.

After 150–200 analyses, exhausted lead peroxide may cause considerable errors, which are observed in both absorption tubes through larger or smaller fluctuations in weight. In this case, the tubes must be refilled.

Increase in weight of both absorption tubes may easily occur in blank tests if faulty rubber tubing is used. The connecting piece between the combustion tube and the calcium chloride tube is especially liable to be affected, and therefore requires particular attention. Also the completely matured rubber tubing absorbs moisture from the air and gives it up again on heating. Remedy: When the humidity of the air is rather high, keep the tubing in a desiccator containing calcium chloride, without evacuation. Positive errors, particularly in the calcium chloride tube, are also due to treatment with too much glycerine.

Negative values for the blank determination may be due to the calcium chloride or anhydrone and soda-lime in the U-tube, calcium chloride and soda-lime tubes, especially if the absorption agents are differently active in the individual parts of the apparatus. Calcium chloride is scarcely a source of error in freshly filled absorption tubes, but if soda-lime is too dry [1] it removes the last traces of moisture from the gases passing over it. Remedy: Correct moistening as on p. 36.

Equilibrium of the calcium chloride may be injured if the U-tube becomes proportionately damp after long use, and gives up water to the calcium chloride tube in the blank test ; newly filled soda-lime tubes also increase in weight. Remedy: Refilling of the U-tube, calcium chloride and soda-lime tube.

In order to avoid errors and disappointments through the absorption agents, when the calcium chloride tube is refilled the U-tube and the soda-lime tube should also be charged with the same

[1] See p. 36.

preparation of calcium chloride or anhydrone. After leaving the apparatus idle for some weeks, all absorption agents should be renewed. After longer interruptions in the work, the tube should be ignited in a current of oxygen for at least three hours before proceeding to the first determination.

Some sources of error may be observed, which, even with irreproachable blank tests, lead to analytical results which cannot be used.

A spent filling of soda-lime always leads to too low carbon values after the addition of 150–200 mgm. of carbon dioxide, in spite of good blank tests. On freshly filling, see p. 42.

Spent lead peroxide can lead to false analytical results before it is detected as unusable in the blank test and in the test analysis with a substance containing carbon, hydrogen, and oxygen only. The strongly attacked lead peroxide or silver no longer retains quantitatively all the oxides of nitrogen in the combustion of organic substances containing nitrogen or halogen.

One can, however, protect oneself against such cases in a simple way, by following a blank determination with a test analysis on substances containing nitrogen or halogen. It is important to check older combustion tubes which have been used for about 100 combustions of substances containing halogen or nitrogen and in which further substances of the kind may be analysed. Then, if the lead peroxide is spent, this is detected in the usual test analysis and in the blank test.

Besides the well-known phenomenon of temporary absorption of water, lead peroxide loses water if it is washed by a current of dry air or oxygen for two to three hours at the temperature of boiling Dekaline, to ignite the tube. During the first analyses, therefore, the lead peroxide reabsorbs the water lost ; this causes too low a value for hydrogen. These observations have already been made by M. Boetius,[1] on the use of phosphorus pentoxide as absorption agent. Also, by the use of calcium chloride or anhydrone I have finally obtained too low values for hydrogen throughout, even if they are still within the limits of permissible error, for all test analyses and duplicate determinations, the first of which was carried out in the analysis series and the second after an interval of half an hour in which the tube was heated in a stream of oxygen.

For this reason I have for some years been in the habit of carrying out a normal combustion with an unweighed substance (*e.g.*, 5–7 mgm. of alizarine) and unweighed absorption apparatus, in a tube heated for one to two hours, in order to establish equilibrium

[1] M. Boetius : "On the Sources of Error of the Microanalytical Determination of Carbon and Hydrogen by Pregl's Method," p. 56, 1931.

throughout the whole system before making the first test analysis. The hydrogen value of the first test analysis is usually more or less inaccurate ; it may also be too high if, *e.g.*, the tube is heated for too short a time and the rubber tubing gives up water absorbed after being disconnected for some time.

Combustion of Metallic Organic Compounds

With Simultaneous Determination of the Residue

The following determinations of metals could almost always be carried out more accurately if it were a question of a simple metallic salt, *e.g.*, of carbonic acid. If, however, the metals are present as complex salts, phenomena must be considered which do not always permit irreproachable weighing. In such cases the result is checked by a determination of the residue according to p. 136.

(*a*) Silver, gold, and platinum may be determined as metals even in the presence of chlorine, by simply weighing the residue. The combustion must be carefully carried out, especially at the beginning. (*b*) Organic salts of iron, chromium, aluminium, copper and tin give good results for the metals weighed as oxides (Fe_2O_3, Cr_2O_3, Al_2O_3, CuO, and SnO_2) after heating strongly in the current of oxygen. Magnesium and lead can similarly be weighed as MgO and PbO if not heated too strongly.

Without Weighing the Residue

(*a*) Compounds of *alkalies and alkaline earths* are best weighed in an old platinum boat and covered with about five to eight times their amount of the purest potassium dichromate, which is finely powdered and kept in a desiccator over phosphorus pentoxide after previously being heated to its melting-point. In the combustion, the tube is protected from contamination due to spirting of the dichromate by pushing the boat into a cylinder of platinum foil (p. 51), and this is boiled in hot water immediately after the analysis.

If potassium dichromate has been omitted in burning a salt of an alkali or an alkaline earth, the residue is weighed and converted into sulphate as on p. 138. From this, the carbonate in the residue is calculated and the corresponding amount of carbon dioxide added to that weighed in the soda-lime tube.

If siliceous compounds should present difficulties in direct combustion, it is advisable to analyse these also in fused potassium dichromate.

(*b*) *Organic metallic compounds*, which on combustion yield oxides

of several stages of oxidation, or such as give more or less liquid oxides.

Among the former may be included salts of manganese, cobalt and nickel, the carbonates of which are decomposed at the temperature of combustion and hence can be analysed without addition. The metals, however, cannot be determined by weighing the residues, because, *e.g.*, manganese heated in a current of oxygen forms both MnO_2 and Mn_3O_4, whilst cobalt and nickel give mixtures of higher and lower oxides.

Zinc oxide is too volatile for the determination of zinc in the residue to be possible ; molybdenum forms its volatile trioxide.

Compounds of arsenic, antimony, and bismuth should not be burnt in new tubes, because their oxides attack the filling too much. According to the investigations of F. Hernler [1] and M. Furter,[2] rather large amounts of mercury injure the tube filling very considerably. On the one hand, too high values are obtained for hydrogen, because the mercury distils through the neck into the calcium chloride tube. On the other hand, the filling is poisoned, which leads to a deficiency of carbon in the subsequent analyses.[3]

Metallic mercury brought into the tube filling in smaller amounts has no noticeable influence on the carbon and hydrogen values. I was convinced of this at the Pregl Institut. Naturally, one prefers to use older tubes for the combustion of mercury salts. If a substance containing mercury is to be analysed, gold wire or gold foil is brought into the neck of the combustion tube and left there for the following ten to fifteen analyses.

New Methods for the Determination of Carbon and Hydrogen

In an endeavour to make the determination of carbon and hydrogen independent of sources of error such as appear occasionally in carrying out analyses, A. Friedrich [4] has developed a method which diverges from F. Pregl's in the following points :

For measuring the speed of the gas, the pressure regulators are replaced by an apparatus on the principle of E. H. Riesenfeld's [5] gas-speed meters. The U-tube is provided with stop-cocks, in order to protect the absorption agents (calcium chloride and soda-lime) from moisture while the apparatus is not in use. The substance is burnt in tubes of resistance-glass, whereby a discharge occurs of

[1] F. Hernler : *Mikrochem.*, Pregl Festschrift, 1929, 154.
[2] M. Furter : *Mikrochem.*, **9**, 27 (1931).
[3] A. Verdino : *Mikrochem.*, **6**, 5 (1928).
[4] A. Friedrich : *Mikrochem.*, **10**, 329 (1932).
[5] E. H. Riesenfeld : *Chem. Ztg.*, **42**, 10 (1918).

the platinum contacts which are introduced instead of the fixed oxidation layer. For the combustion of substances containing nitrogen, sulphur, and halogen, two boats containing lead peroxide are brought into the tube. The choking plug is retained.

When the absorption tubes are weighed full of oxygen, as in B. Flaschenträger's [1] method, they can be closed by stop-cocks. They do not possess any side tubes drawn out to capillaries, therefore the stream of gas meets with no resistance and the analysis can be carried out in a shorter time. The Mariotte flask is superfluous.

J. B. Niederl and J. R. Meadow [2] share a method for the determination of carbon and hydrogen on a weight of less than 1 mgm. of substance.

The combustion is carried out according to F. Pregl, the water collected in calcium chloride as above ; the carbon dioxide, however, is led into a solution of barium hydroxide and the precipitated barium carbonate dried and weighed. Since the barium carbonate is sixteen and a half times as heavy as its carbon content, the equivalent proportions are very satisfactory. As the variations between test analyses, up to now, have been greater than usual ($\pm$ 0·7 per cent.), this method cannot be finally judged as yet.

P. Kirk [3] replaces Pregl's layer of lead chromate and copper oxide by " catalytic pyrolusite," the temperature of which is kept at 400° C. ; this oxidation layer remains satisfactory for about eighty analyses. The absorption tubes are weighed full of oxygen ; results are approximately equal to those of F. Pregl.

J. B. Niederl and B. Whitman [4] have suggested replacing the lead peroxide by copper and burning the substance in the stream of nitrogen. The substance is introduced into the tube in the boat or in a cupric oxide mixture, as in the volumetric determination of nitrogen. The tube filling consists of cupric oxide, lead chromate, silver, and an intermediate layer of reduced copper. Water and carbon dioxide are weighed in nitrogen in Pregl's absorption tubes. Because the investigation is still being carried out, a fuller account will not be given.

Volumetric Determination of Carbon and Hydrogen by J. Lindner's Method [5]

J. Lindner has modified F. Pregl's method by titrating the water and carbon dioxide formed. The products of combustion pass from the combustion tube through a mercury valve into a " phos-

[1] B. Flaschenträger : *Z. angew. Chem.*, **39**, 720 (1926).
[2] J. B. Niederl and J. R. Meadow : *Mikrochem.*, **9**, 350 (1931).
[3] P. L. Kirk and Ag. McCalla : *Mikrochem.*, **12**, 87 (1933).
[4] J. B. Niederl and B. Whitman : *Mikrochem.*, **11**, 274 (1932).
[5] J. Lindner : *Z. analyt. Chem.*, **66**, 305 (1925).

phine apparatus." At about 105° C. the following reaction occurs between water and the volatile naphthyloxychlorphosphine :

$$R - POCl_2 + H_2O = R - PO_2 + 2HCl.$$
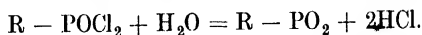
For every molecule of water, two molecules of hydrochloric acid are titrated. The carbon dioxide is absorbed in barium hydroxide solution and titrated. While the carbon values are of the same accuracy as Pregl's, the hydrogen may be determined to ± 0.05 per cent. and even more accurately.[1]

J. Lindner[2] has in view a comprehensive description of the use of copper instead of lead dioxide. Wt. of substance used $= 10$–15 mgm.

Determination of Carbon in the Wet Way

Although carbon can be determined more accurately in the wet way, the chemist still gives up all claim to hydrogen values except in very exceptional cases. The use of the following methods should therefore still be limited to physiological material.

H. Dieterle[3] oxidised the organic substance with sulphuric acid and potassium dichromate. The products of decomposition arrive in the stream of oxygen in an ignited layer of potassium chromate and copper oxide, where they are completely oxidised to carbon dioxide. The carbon dioxide is determined gravimetrically in a Blumer tube.

H. Lieb and H. G. Krainick[4] use, besides sulphuric acid and potassium dichromate, the catalytically active silver dichromate already recommended by J. Lindner, and lead the products of combustion over red-hot platinum contacts into barium hydroxide solution, in which the carbon dioxide is very accurately determined volumetrically as in J. Lindner's method. In the application of this method to biological liquids, E. Schadendorff and M. K. Zacherl[5] have made an alteration in the reaction vessel which makes it possible in the system free from carbon dioxide, to burst the capillaries of the material with a stamp.

The application of this process could apparently be extended by the principle of R. Kuhn and F. L'Orsa.[6] According to this process, the hydrogen can often be calculated from the "Total carbon balance," which is obtained from the titration of the unused chromic acid together with the weighed carbon dioxide.

[1] *Cf.* H. Wieland and G. Hess : "On the constitution of the poison of the native toad."
[2] J. Lindner : *Ber. dtsch. chem. Ges.*, **65,** 1696 (1932).
[3] H. Dieterle : *Arch. Pharmaz.*, **262,** 35 (1924).
[4] H. Lieb and H. G. Krainick : *Mikrochem.*, **10,** 99 (1932).
[5] E. Schadendorff and M. K. Zacherl, *Mikrochem.*, **10,** 99 (1932).
[6] R. Kuhn and F. L'Orsa : *Z. angew. Chem.*, **44,** 847 (1931).

II. THE GAS-VOLUMETRIC DETERMINATION OF NITROGEN BY F. PREGL'S METHOD (MICRO-DUMAS) *

History and Principles

In F. Pregl's first micro-combustion tubes the reduced copper spiral was found at the end of the combustion tube as in the macro-Dumas method. This " quick empirical method " was carried out in about twenty minutes and yielded accurate nitrogen values to the expert, if 10 per cent. were deducted from the gas volume collected. This excess was originally ascribed by F. Pregl solely to the space taken up by the 50 per cent. caustic potash adhering to the walls of the azotometer. More recent investigations showed, however, that the volume taken up by the 50 per cent. caustic potash was about 2 per cent. of the measured volume.[1] The remaining 8 per cent. of the excess volume was traced to other causes. If the amounts of material were increased and the combustion carried out very quickly, a gas was formed which burned with a bluish flame and which gave the carbon monoxide reaction with hæmoglobin on leading into a dilute solution of blood. This result indicated that the unexplained 8 per cent. of the gas volume must be due to carbon monoxide and possibly also to some other associated gas.

Besides the too short period of contact of the gas with the red-hot tube filling in the empirical method, the reduced copper spiral might also be the cause of the increase in volume of the gas. If, that is, a copper spiral were heated in a tube through which carbon dioxide is passed slowly, which supplies " ideal " micro-bubbles to the azotometer, an increase in the size of the rising bubbles can easily be detected. This phenomenon is due to a disturbance of the equilibrium between carbon dioxide on the one hand and carbon monoxide and oxygen on the other.

As, however, F. Pregl placed the reduced copper in the middle of the tube, filled up from here to the attached neck with a larger amount of copper oxide, and finally allowed the combustion tube to project for some centimetres beyond the tube-burner, a fall in temperature occurred in the projecting portion of the tube, which facilitated the complete oxidation of any carbon monoxide. In the azotometer, then, only minute bubbles rise from the heated tube, which do not produce any measurable volume of gas during an experiment lasting half an hour.

On the assumption that the diameter of the ascending bubbles is about 0·2 mm.—with properly manipulated Kipp apparatus still smaller bubbles are easily obtained—then 250 such bubbles have a

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[1] On the correction of 2 per cent., *cf.* p. 72.

volume of 0·001 c.c. If such bubbles rise at the rate of one per
second, a volume of 0·014 c.c. is only produced in an hour.

These phenomena had not entirely escaped the attention of
former careful observers such as E. Ludwig and G. Goldschmiedt,
and these workers and others already recommended the addition of
an oxidised roll of copper gauze behind the reduced copper spiral
while carrying out nitrogen determinations by the method of
Dumas. These experiments caused the copper spiral to be moved
from the end of the tube and the reduced copper to be placed in
the interior of the tube.

We must further reckon with the fact that during the combustion
of organic substances nitrous oxide may also be obtained. This
would not in itself affect the analytical result, as nitrous oxide takes
up the same volume as the nitrogen which it contains. Since,
however, at very high temperatures nitrous oxide dissociates into
nitrogen and oxygen according to the equation :

$$2N_2O = 2N_2 + O_2,$$
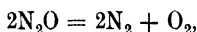
unless the oxygen produced is completely absorbed some of it will
collect in the azotometer. This is prevented by using proportionally
large quantities of reduced copper at the hottest part of the
combustion tube.

The further observation, that if a tube filled with copper oxide
only is alternately heated and cooled, rather larger bubbles will be
formed in the azotometer, which will again become smaller on
cooling, also appears to depend on a perceptible dissociation of
carbon dioxide. F. Pregl dealt with this phenomenon when he
allowed the tube to project 40 mm. beyond the tube burner. The
fall in temperature thus resulting completely prevented the forma-
tion of carbon monoxide.

The question of whether it is better to arrange the copper spiral
at the end, as recommended by J. Dubsky, or in the middle, as
recommended by F. Pregl, has been systematically investigated by
H. Fischer, who definitely expresses himself in favour of the middle
position for the copper spiral. F. Böck and K. Beaucourt have
thoroughly studied the problem experimentally and come to the
conclusion that the tube-filling with copper in the middle appeared
to be justified as a precautionary measure. F. Hernler states that
the experimental displacement of the copper spiral to the end of the
combustion tube exercised an unfavourable influence on the results.

For the period of combustion, F. Pregl has prescribed a bubble
frequency of not more than one in one and a half seconds. This was
the result of knowledge accumulated very painstakingly on the
reactions of combustion, the passing of the gases over the red-hot

tube filling, the length of the filling and cross-section of the tube, and the development of gas.

The velocity of the gas thus determined should never be exceeded if satisfactory results are to be obtained.

B. Flaschenträger[1] has observed that coarse copper oxide is occasionally the cause of high nitrogen values, which are traced back to the enclosure of air. He recommends leaving the coarse copper oxide of the movable filling in the tube for several analyses. Shintaro Ogawa[2] confirmed this observation and removed the air from the hollow spaces by evacuating the tube and subsequently filling with carbon dioxide, by the method of E. Berl and H. Burkhart.[3]

F. Hernler[4] allows the coarse and fine copper oxide to cool in a Kjeldahl flask after repeated evacuations under pure carbon dioxide. According to O. P. Trautz,[5] the " fixed filling " involves no source of error through enclosed or adsorbed air, if the tube is always kept under a slight excess-pressure of carbon dioxide. The " movable filling," on the other hand, if it consists as usual of copper oxide which has been ignited and cooled in air, and the carbon dioxide taken from the Kipp apparatus, form a nearly constant source of gas, because for every analysis about the same volume of carbon dioxide and also the same amount of copper oxide is used. It is a question of about 0·003 to 0·006 c.c. of nitrogen for each analysis, that is about 1 per cent. of the volume of nitrogen which is normally obtained. Second to this approximately constant correction there is still to be undertaken a correction proportional to the volume of nitrogen collected, which O. P. Trautz has determined to be 0·8 per cent. It is composed of 0·5 per cent. for the volume diminution caused by the 50 per cent. caustic potash solution, which has been determined gravimetrically and volumetrically, and 0·3 per cent. for the vapour tension of the caustic potash solution. A. Friedrich[6] evacuates the filled tube by the stop-cock of the intermediate pieces and then allows the carbon dioxide from the Kipp apparatus to stream in. After this has been repeated three times he obtains the correct nitrogen value, when only 0·5–1 per cent. of the total volume is deducted for the volume diminution caused by the 50 per cent. caustic potash solution.

Some experiments to free new tube filling from enclosed gas by repeated evacuations followed by admission of carbon dioxide

[1] B. Flaschenträger : *Z. angew. Chem.*, **39**, 717 (1926).
[2] S. Ogawa : *Sci. Rep. Tohoku Imp. Univ.*, **16**, 667 (1927) ; C. 1928 I., 380.
[3] E. Berl and H. Burkhart : *Ber. dtsch. chem. Ges.*, **59**, 897 (1926) ; *cf.* Böck and Beaucourt : *Mikrochem.*, **6**, 69 (1928).
[4] F. Hernler : " Pregl Festschrift," 1929, 143.
[5] O. P. Trautz : *Mikrochem.*, **9**, 300 (1931).
[6] A. Friedrich : *Mikrochem.*, **10**, 355 (1932).

have confirmed the observations published by O. P. Trautz and A. Friedrich.   If, however, the coarse and fine copper oxide, ignited in air as by Pregl's method, were no longer evacuated for the following test analyses, then after the second to third analysis the correction of 2 per cent. must again be introduced, which afterwards remains constant.   All these investigations confirm the volume correction of 2 per cent. experimentally ascertained by F. Pregl.   It is, however, not only a question of the volume diminution due to the caustic potash solution.   About 1–1·2 per cent. is due to the air retained by the copper oxide of the movable filling, which is independent of the volume of nitrogen obtained.   The correction of 2 per cent. should be retained.   It is certainly said to be exact only when the " constant error " amounts to 1·2 per cent. of the gas volume, that is when 0·3–0·5 c.c. of nitrogen are obtained, as described by F. Pregl.   The results must be incorrect if we deduct 2 per cent. equally for nitrogen volumes under 0·2 or over 0·6 c.c. In the first case, too high volumes of nitrogen are to be expected, and in the second, too low ones.   Practical experience from numerous analyses is in harmony with this.

For the first determinations with azobenzene, combustion tubes with new fixed fillings give results too high by about 0·3–0·5 per cent., which are traced back to the gases adsorbed.   But if the tube is kept permanently filled with carbon dioxide, the values obtained after two or three analyses are excellent.   Before the first test analyses it is advisable, therefore, to ignite new tubes strongly three times in a slow stream of carbon dioxide, which passes at the rate of one bubble per second.   In recent years, about ten tubes pretreated in this simple manner gave nitrogen values for the first test analyses on azobenzene which differed from the theoretical ones by less than ± 0·1 per cent.

On account of its reliability and ease of manipulation, F. Pregl's micro-Dumas method is no longer the monopoly of the specialist, but it has also been applied successfully in laboratories where analyses are only carried out incidentally by the working organic chemist, and it is to-day taught in the curriculum of many Universities and High School Institutes instead of the macro-Dumas method.

The Kipp Generator

Because the carbon dioxide must be extremely pure in order that it may be absorbed by 50 per cent. caustic potash with a scarcely visible residue (micro-bubbles), attention must be paid to the filling and manipulation of the Kipp generator.

Preparation of the Marble.   Very pure, finely porous marble, which yields sufficiently small micro-bubbles very soon after etching

with hydrochloric acid, cannot be obtained everywhere. By the following process, perfect micro-bubbles have been obtained from all commercial marbles used in the last five years :

The marble is broken up to the size of hazel nuts, etched with dilute hydrochloric acid, washed, covered with water, and boiled for ten minutes. After cooling, it is placed in a large desiccator and covered with calcium chloride solution, which has been syphoned off from the exhausted Kipp generator and neutralised with marble.[1] The desiccator is now evacuated, by means of the water-pump, to remove the air from the pores of the marble. If, after half to one hour, no more bubbles escape from the marble, the vacuum is slowly abolished, when the pores fill with calcium chloride solution. In order to remove the last traces of air, it is again evacuated for thirty minutes, after which the marble is ready for filling the generator.[2]

In order to remove all the air from the 2-litre Kipp apparatus as quickly as possible, the inner end of the tube with the glass stop-cock is connected with a hooked glass tube by a short piece of rubber tubing, so that the gas can be drawn from the highest point of the middle bulb (see Fig. 22).

The middle bulb of the generator is filled up to the tubulus with the treated marble. Short glass rods are better than leather or rubber discs for preventing the marble from dropping into the lower bulb.[3] Then the rubber stopper, moistened with glycerine, is placed in the tubulus.

The carbon dioxide is delivered to the combustion tube from the highest part of the middle bulb, through the tube with stop-cock. On to this tube, past the tap, it is advisable to fuse a bulb-shaped enlargement, which passes into a glass tube of the same bore. The bulb is filled with cotton-wool, which keeps back the mist of hydrochloric acid carried over by the carbon dioxide. The enlarged glass attachment of the thick-walled capillary *R* (thermometer tube) is connected to the delivery tube of the Kipp apparatus by means of a very tight piece of pressure tubing, about 100 mm. long, which is moistened with glycerine. The two tubes are in contact ; they are of the same diameter. To prevent cracking of the rubber tubing, it is wrapped round with several strips of paper. The other end of the thermometer tube, which forms a tapered capillary, is fitted into the mouth of the combustion tube through a rubber stopper. One litre of pure, fuming hydrochloric acid diluted with

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[1] The solution for filling new Kipp generators is prepared from very dilute hydrochloric acid and marble.

[2] A very similar pre-treatment of the marble is recommended by A. Friedrich : *Mikrochem.*, **10**, 358 (1932).

[3] The firm of P. Haack has recently produced a perforated glass cylinder, which is drawn over the central tube at the bottom of the middle bulb.

the same volume of tap water is used for filling the lower bulb and about half or one-third of the upper one.

De-aeration. If the cock H_1 (Fig. 22) is then opened, air is
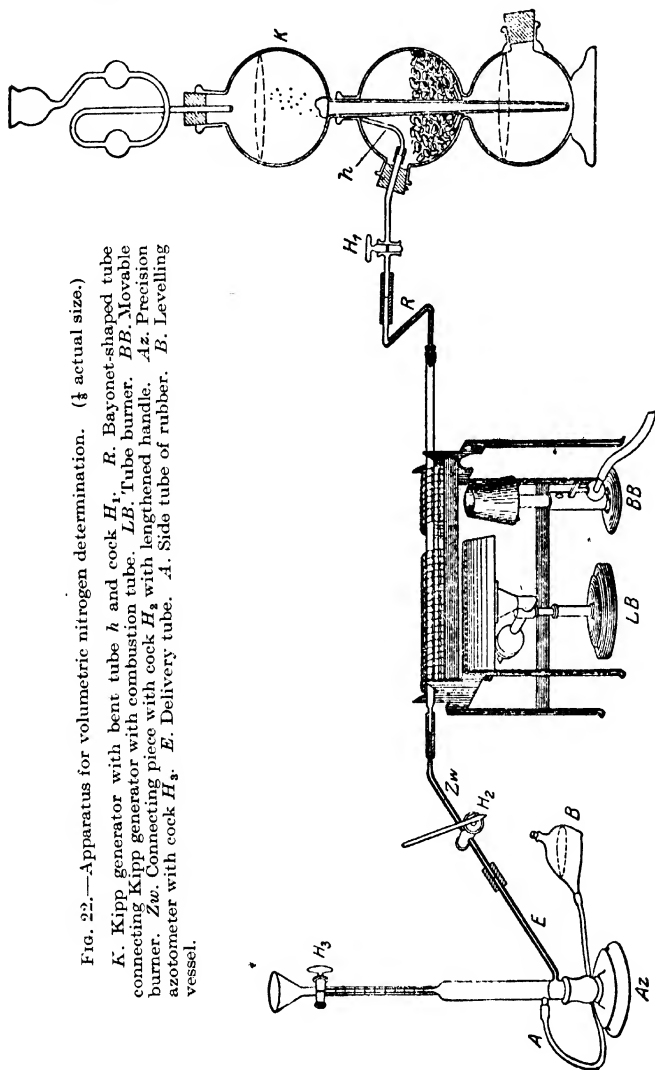
FIG. 22.—Apparatus for volumetric nitrogen determination. (⅓ actual size.)

K. Kipp generator with bent tube *h* and cock H_1. *R.* Bayonet-shaped tube connecting Kipp generator with combustion tube. *LB.* Tube burner. *BB.* Movable burner. *Zw.* Connecting piece with cock H_2 with lengthened handle. *Az.* Precision azotometer with cock H_3. *E.* Delivery tube. *A.* Side tube of rubber. *B.* Levelling vessel.

expelled from the middle bulb and carbon dioxide is liberated by the acid which enters. The gas which is then obtained from the apparatus corresponds by no means to the high degree of purity required for the nitrogen determination, for the acid still contains a large amount of air in solution which mixes with the carbon dioxide

developed. Two or three lumps of marble of the size of filberts are therefore dropped into the upper bulb. These remain in the central tube and there develop carbon dioxide in abundance. The air is thus completely removed from the hydrochloric acid in the upper bulb if fresh acid is admitted to the upper bulb by repeatedly opening and closing the cock. A freshly assembled Kipp apparatus, which has been treated as just described, does not as a rule deliver gas of the necessary high degree of purity ; it is best to allow it to stand for two or three days, after which fresh lumps of marble are thrown into the upper bulb to repeat the de-aeration. Obviously, new generators contain large amounts of air, not only on the surfaces of the glass, but also in the rubber stoppers, and this is only liberated after being exposed for days to the atmo-sphere of carbon dioxide. In the case of apparatus which has already been used and is immediately refilled after being completely cleaned, one day at most is sufficient.

The constant contact of the hydrochloric acid in the upper bulb leads to admission of air into the completely de-aerated Kipp generator. This makes it necessary to de-aerate it again in the manner described, at least after standing overnight.

A modification introduced by F. Hein [1] enables us to avoid the constant contact of the acid with the atmosphere (Fig. 23). On the one hand, the upper bulb is closed against the air of the room by a mercury valve, and, on the other hand, the gas-filled portion of the middle bulb is connected with the upper bulb

Fig. 23.—Hein's modifi-cation of Kipp genera-tor with mercury valve.

through a tube which includes a glass cock. When competently handled, this arrangement enables air-free carbon dioxide to be available much more rapidly and, to a certain degree, to be stored. Its manipulation, however, requires care and experience.

Preliminary Examination of Micro-bubbles. After the necessary time of standing it is found that the carbon dioxide is almost completely absorbed by the potassium hydroxide solution, so that the bubbles vanish, leaving a scarcely perceptible trace. If the micro-bubbles are led for half an hour into the azotometer, at a rate of one bubble per second, scarcely any bubbles should be visible below the tap H_3.[2]

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[1] F. Hein : *Z. angew. Chem.*, **40**, 864 (1927).
[2] On the rapid examination of micro-bubbles, see under " Carrying-out the combustion," p. 82.

Renewal of the Acid. If the acid has become weakened by use, it is advisable to empty the generator in such a manner that no air enters the middle bulb. This is best done by syphoning the acid from the upper bulb after the acid has risen into the same, or it may be done by running off the acid through a glass cock which is fixed into the tubulus of the lower bulb. Half of the acid drawn off is replaced by the same volume of pure concentrated hydrochloric acid, and this acid, containing calcium chloride, is used for refilling the generator. The calcium chloride content of this mixture gives it the advantage that its coefficient of absorption for air is considerably lower than that of a mixture of acid and pure water.

Reagents

Copper Oxide and Metallic Copper. Two forms of copper oxide are used for filling the combustion tube ; they are kept in wide-mouthed bottles with ground-in stoppers. They are : (1) *Wire-form copper oxide*, which is crushed in a mortar before use in order to obtain pieces 4–5 mm. long on an average, and (2) *finely-divided copper oxide*, which is obtained by grinding the wire-form product in a mortar and passing it through a sieve. Very finely-powdered copper oxide, or the precipitated product, cannot be used, as they offer too much resistance to the gas current.

The metallic copper may be prepared by reduction with hydrogen when filling the tube. Where large quantities are used it is advisable to reduce a large amount of the wire-form copper oxide completely with hydrogen in a combustion tube and to maintain a stock of this material for tube-fillings.

The filling removed from the tube after every analysis is kept in a wide-mouthed bottle, and amounts collected from about five fillings are ignited in a nickel dish for fifteen minutes. After cooling, the coarser and finer portions are separated by means of a fine-meshed sieve and stored separately, for further analyses.

Non-frothing Fifty per cent. Potassium Hydroxide Solution. It is absolutely necessary, in order to read the small gas-volumes, that the level of the 50 per cent. potassium hydroxide solution in the micro-azotometer should be absolutely free from foam. Two hundred grams of potassium hydroxide in sticks are dissolved in 200 c.c. of water and treated with 5 gm. of finely-powdered barium hydroxide. After shaking, the liquid is allowed to stand for a quarter of an hour, to allow most of the precipitated barium hydroxide to settle. It is then filtered through ordinary dry filter paper, and stored in bottles with rubber stoppers.

Filling and Preparation of the Combustion Tube

A combustion tube 500 mm. long, provided with a neck, is used. Into this an ignited wad of asbestos is pushed up to the neck with a glass rod and there compressed to a length of about 5-6 mm. Wire-form copper oxide is then introduced, to a length of 130 mm., and this permanent filling is held in position by means of a gently compressed asbestos plug.

A short roll of wire gauze, 40 mm. long, is now passed over the tube, which is placed on a combustion stand, as in the determination of carbon and hydrogen (p. 24). A layer of copper oxide about 40 mm. long is then reduced to metallic copper by heating, beginning at the asbestos plug in the middle and moving the burner slowly up to the neck, in a current of hydrogen, which has been passed through potassium permanganate solution.

It is advisable, before using the tube with its permanent filling for the first time, to preheat it three times in a slow stream of carbon dioxide and allow it to cool in this. When not in use, it is left on the combustion stand, still connected with the Kipp generator, so that it contains carbon dioxide under pressure. By using this precaution, the copper oxide need not be freshly reduced or renewed until several hundred analyses have been carried out. By always keeping ten tubes filled with carbon dioxide under pressure, the further advantage is gained that air is more quickly removed before analysis, and for both reasons the use of the Kipp generator is favoured rather than heating sodium bicarbonate ; the latter has the further disadvantage that volatile substances undergo considerable loss on passing over them a current of warm carbon dioxide.

The permanent filling is followed by a movable filling which is renewed for each analysis.

The permanent filling can also be followed immediately by coarse copper oxide (before the reduced copper), which projects 3-4 c.c. over the tube burner and which must be renewed at once if it shows even partial reduction. This filling protects the permanent filling, to a certain degree, from the enclosure of air after ignition ; therefore the time of passing carbon dioxide over before the combustion is shortened. The movable filling consists, therefore, of the fine copper oxide only.

The Precision Microazotometer

The zero point of the scale is at the cock. The scale begins at about 0·05 c.c. and usually reaches to 1·2 c.c., sometimes to 1·5 c.c. The spaces between the divisions correspond with 0·01 c.c., and the volume can easily be estimated to 0·001 c.c., particularly with the help of a reading lens as supplied by Köhler, Leipzig. The

separate scale divisions take up three-quarters of the circumference of the tube, making it possible to read the volume without error due to parallax by so placing the lens that the scale divisions coincide on both sides.

P. Haack's microazotometers are calibrated with mercury from the stop-cock to the scale divisions concerned in the inverted azotometer. The convex mercury meniscus in the reversed azoto-meter during calibration and the concave meniscus for potassium hydroxide solution almost coincide. The Physikalische Technische Reichanstalt, Charlottenburg, has determined the difference between them to be about 0·001 c.c., by which the volume found with mercury is smaller.

Azotometers calibrated with mercury in the inverted position are etched with the mark " KOH." Azotometers which are marked " Hg " instead of " KOH " should not be used. They show a difference of about 0·007 c.c. at each scale division, if they are calibrated with mercury in the upright position.

Care should be taken that the inlet tube for the gas should keep its bore of 0·8–1·0 mm. at the position where it is sealed on, and not be widened by sealing. Widening causes too large bubbles. Since the velocity of the combustion is regulated according to the bubble frequency, a considerable error would thus be shown.

Each precision microazotometer is now supplied with a certificate of standardisation, besides a suitable wooden stand and a metal fork, attached by a screw below the funnel, by means of which the levelling vessel may be maintained at the higher level. A further important auxiliary part is the connection Zw with cock H_2, which adjusts the junction at the neck of the combustion tube.

It is advisable that shallow channels should be cut in the tap-barrel with a glass-cutter, which would extend about 4 mm. round its circumference at both ends of the bore.[1]

A. Friedrich [2] recommends providing the connecting-piece with a ground-in joint in which cotton-wool is placed. This prevents water and copper oxide dust passing over into the barrel of the cock, a re-adjustment of the bubble velocity once set up is thus unnecessary.

Filling the Azotometer. The azotometer is cleaned by rinsing it repeatedly with chromic acid in sulphuric acid and water, and is allowed to drain in the inverted position. The levelling vessel, B, which has also been cleaned and dried, is then connected with the

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[1] If water should have collected in the connecting-piece, as may occur especially in the combustion of compounds rich in hydrogen, then it, with the stop-cock, is rinsed with alcohol and dried with the pump, to prevent obstruction of the channels with water produced during the combustion.

[2] A. Friedrich : " Practice of Quantitative Microanalysis," p. 64. Berlin-Vienna. Franz Deuticke.

side tube of the azotometer by means of rubber tubing. It is advisable to bind both ends of this tubing with wire. Mercury is added through the levelling vessel until the level is midway between the inlet tube E and the upper side tube for the attachment of the rubber tube A. Before introducing the 50 per cent. potassium hydroxide solution the cock H_3 is carefully lubricated with a little vaseline. No other lubricating medium must be used, as otherwise the caustic potash begins to foam after a short time. Sufficient of the latter is added to fill the measuring tube and about one-third of the pressure tube.

It may happen that when azotometers which have been filled with perfectly pure mercury and pure 50 per cent. potassium hydroxide solution are first used, the rising gas-bubbles are held at the boundary between the mercury and the potash solution, and only rise after considerable shaking. This occurrence, however, ceases after the first determinations, when sufficient finely-divided copper oxide dust has collected at the boundary between the two liquids.[1]

Carrying out the Determination

Solid substances are now weighed as a rule in weighing tubes with long and short handles (Fig. 24), of H. Lieb and H. G. Krainick ; [2] these can be made very easily. The short-handled tube has a total length of 50–60 mm. The 20 mm. long tube is slightly constricted
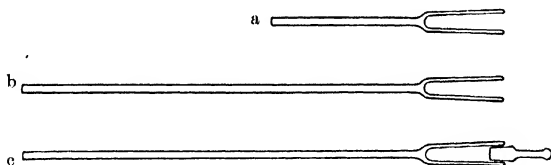
Fig. 24.—Nitrogen weighing tubes of H. Lieb and H. G. Krainick. (*a*) With short handle. (*b*) With long handle. (*c*) With ground-in stopper for weighing hygroscopic substances. (Actual size.)

at the handle ; at the upper end the diameter is 3·5 mm. The weight should be a few tenths of a milligram over 500 mgm. A 500 mgm. weight serves as a counterpoise, so that complete weighings can be undertaken with the rider alone. The weighing tubes with long handles are similar in form to the short ones. The counterpoise of the heavier tube is just over 1,000 mgm. For weighing, it is placed in the hook of the balance suspension. The long-handled tube is indispensable for weighing substances for the determination of the

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[1] C. M. Nickols, *Ind. Eng. Chem. Analyt. Edn.*, **5**, 149; C 1933 I., 3973, makes the bubbles rise by the addition of mercuric oxide.
[2] H. Lieb and H. G. Krainick : *Mikrochem.*, **9**, 367 (1931).

active hydrogen of the isopropylidene and acetyl groups. When residues stick to the tube, it is cleaned with alcohol and acetone, dried by drawing once through a non-luminous flame, wiped with chamois leather and placed near the balance. For weighing hygroscopic substances the tubes possess ground-in stoppers (Fig. 24,c).

Oily and syrupy substances are weighed, as for the carbon and hydrogen determinations, in porcelain or platinum boats and covered to the edge of the boat with fine copper oxide. Liquids are weighed by the method described for carbon and hydrogen determinations (p. 51). Here also, a crystal of potassium chlorate is fused in the capillary and the capillary closed. Low boiling liquids are weighed by J. Pirsch's [1] method, which avoids the use of unnecessarily large capillaries, because the nitrogen of the air enclosed is obtained in the nitrometer after the combustion.

As a rule 2–5 mgm. of material are weighed out, which should yield a volume of 0·3–0·5 c.c. of nitrogen. For substances very rich in nitrogen the weight must be reduced to about 1·5 mgm. and the weighing carried out with the greatest possible accuracy. Substances which contain less than 2 per cent. of nitrogen and those which are examined for traces of nitrogen, are exceptions ; in order that in such cases also the limiting error of 0·2 per cent. shall not be exceeded, the weight used should be increased to 8–10 mgm., but not more.

After the weight of the tube filled with the material has been noted, the material is emptied into a mixing tube. The mixing tube can best be made from a micro-bomb tube (p. 105), which is cut down to a length of 70–80 mm. ; the sharp edges are carefully rounded with fine glass-paper and allowed to become smooth in the flame.

For transferring the material, the weighing tube is held by the handle in a chamois leather in the left hand and taken from the balance with the mouth held obliquely uppermost. The mixing tube, from the top third of which copper oxide dust has been removed by cotton-wool, is held horizontally in the right hand. The mouth of the weighing tube is pushed 1–2 c.c. into the mixing tube and inclined until the material falls into the latter. If the substance sticks fast, the weighing tube is gently tapped or rotated. The two tubes are then again held horizontally, one inside the other, and the traces of substance gently tapped into the mixing tube. The substance is covered with 20 mm. of fine copper oxide and the mixing tube closed with a non-porous cork.

After five minutes the emptied weighing tube is weighed again to ascertain the amount of substance used.

[1] J. Pirsch : *Ber. dtsch. chem. Ges.*, **65**, 865 (1932) ; *cf.* p. 52.

Filling the Combustion Tube

The rubber tubing [1] is removed from the neck, the combustion tube is disconnected at *R* from the Kipp generator, and the movable filling emptied into a stock bottle. Fresh wire-form copper oxide from the wide-mouthed bottle is introduced, to a length of 90–100 mm.,[2] by scooping it out with the combustion tube. Finely powdered copper oxide is poured on top of this filling, to a length of 5–10 mm. This prevents any particles of the material to be analysed from falling into the coarse filling, and thus undergoing premature combustion. The material covered with copper oxide is intimately mixed with this in the mixing tube by vigorous shaking. After removing the cork by turning it with constant tapping, the mixture is allowed to slide into the tube through a filling funnel (Fig. 25), which is prepared by drawing out a test-tube in the middle to a diameter of 5 mm. and a length of 60 mm. To remove the remaining material from the mixing tube, about the same amount of fine copper oxide as previously used is scooped out of the stock bottle with the open end of the mixing tube, which is closed again with the cork, well shaken, and poured into the combustion tube. This operation is repeated three times more. After this has all been transferred, there is in the combustion tube a layer of fine copper oxide, a length of about 90 mm. in all. After removing the filling funnel, a further layer of 10–20 mm. of coarse copper oxide is brought into the tube.

Fig. 25.—Filling funnel. (Actual size.)

If the substance is weighed in the platinum boat, 50 mm. of fine copper oxide is similarly inserted on the coarse copper oxide. The boat is allowed to slide along the tube on to this layer, and is covered over again with a 40–50 mm. long layer of fine, and finally with a 10–20 mm. layer of coarse, copper oxide.

Capillaries of liquid are protected by pushing them into a copper roll of wire gauze, 40 mm. long and 5 mm. inner diameter, immediately after breaking off the handle and tip. The whole is allowed to slide into the tube and a layer of coarse copper oxide is quickly poured on it as usual.

After covering the combustion tube with a close-fitting roll of wire gauze 150 mm. long for the tube burner and a similar one 40 mm. long for the movable burner, the tube is placed on the combustion stand so that the neck still projects 40 mm., and the

[1] Absorption-tubing for the carbon and hydrogen determination, p. 25.
[2] If the permanent filling has been placed against the fine copper oxide, this filling is omitted.

open end of the tube is connected witht he delivery tube of the Kipp generator by means of a perforated rubber stopper.

Carrying out the Combustion

The stop-cock H_1 (Fig. 22) is fully opened and carbon dioxide is passed through the tube for three minutes without insertion of the nitrometer, in order to displace the air from the filling in the tube. Then the azotometer is connected with the neck of the combustion tube by means of the connecting piece, with the cock H_2 open, H_3 of the azotometer is also open. After closing H_2, the azotometer is filled with potassium hydroxide solution by raising the levelling vessel to the height of the cock H_3. After the air from the cock H_3 is replaced by potash, this is closed, the levelling vessel lowered, and by slowly opening the cock H_2 a bubble frequency of three to four bubbles per second is set up. One now begins to heat the tube burner and raises the temperature of the combustion tube to redness.

Apart from observation with a lens, it can be observed whether sufficiently small micro-bubbles are being obtained by their very much slower speed of rising in the azotometer, compared with that of the larger bubbles. In consequence of this, they approach one another in the measuring range of the azotometer and rise slowly and at short intervals from one another in line.

Nothing is gained by passing carbon dioxide for a longer time, and it is actually inadmissible for substances which are appreciably volatile, as in that case vapours of the material would be prematurely burnt and the nitrogen values obtained would be too low. Such observations were repeatedly made by us with nitrosodimethylamine and dinitrotrichlorbenzene. Correct values were only obtained regularly with these materials when the driving out of the air of the tube in the current of carbon dioxide only lasted long enough to produce bubbles of the necessary small size.

When the micro-bubbles are satisfactory, the combustion is begun by first closing cock H_1 of the delivery tube and then fully opening the cock H_2. The small roll of wire gauze is brought over the end of the copper oxide layer and the movable burner is placed below it so that the short roll is in the hottest zone of the flame, which is fully turned on and just non-luminous. The heating of the tube at once gives rise to renewed evolution of gas bubbles into the azotometer, which slackens after some time if the position of the burner is not changed. The levelling vessel is now raised somewhat higher than the cock H_3, the foam taken off in the funnel, and the levelling vessel replaced beside the azotometer, where it remains throughout the combustion. The small roll and the movable burner B are now moved slightly towards the material to be analysed and left at this point until the evolution of bubbles begins to slacken.

The gauze roll and burner are then moved forward again, taking care that bubbles do not enter the azotometer at a rate of more than two bubbles in three seconds. It is easy to avoid exceeding this maximum frequency, firstly, by only moving the burner a few millimetres when in the neighbourhood of the substance analysed, and, secondly, by only moving it forward when the frequency of the bubbles has sunk below the maximum. When the whole section filled with the substance has been heated in the manner described and the bubble-frequency begins to diminish on moving the movable burner further forward, this burner can be moved forward more rapidly until it is immediately behind the tube burner LB.

The cock H_2 is then closed and the cock H_1 of the Kipp generator is opened. Afterwards the cock of the connecting tube H_2 is adjusted very carefully with the help of the long handle with which it is provided, so that again not more than two bubbles rise in three seconds, a lesser speed being maintained by preference.

This adjustment of the stop-cock is probably the only feature of the manipulation in this determination which requires some practice, as it must be so carried out that the above-mentioned velocity of the gas-current is not exceeded for even a few seconds. In such a case it is to be feared that too high nitrogen values would be obtained. The period during which the gas is expelled is utilised by once more igniting the whole length of the tube with the movable burner, during a period of ten minutes. In doing this it is sufficient to place the burner at about four different points within the section to be ignited, from the end of the filling up to the tube burner. The expulsion period can be shortened by extinguishing both burners as soon as the gas bubbles begin to become smaller and increasing the velocity of the current of gas to one bubble per second.

After some time is will be noticed that the gas bubbles appearing in the azotometer gradually become almost as small as those which we described as micro-bubbles at the beginning. It makes no difference, after this, whether the determination is stopped at once or after a few minutes, for, as has been shown by calculation (p. 70), the volume of the micro-bubbles is so small that on allowing them to continue for a longer period the result is not affected unfavourably.

From the beginning of the combustion to this point about thirty to forty minutes have elapsed, according to the quantity of nitrogen in the material. The levelling vessel is now supported in the metal fork near the funnel and the azotometer removed. To prevent access of air to the azotometer, the cock H_2 of the connecting tube is closed before removing the rubber tubing of this tube from the combustion tube. The still warm combustion tube is closed at once with a rubber cap, so that it cools in carbon dioxide under pressure, from the generator.

The azotometer is brought into a sheltered place—in summer it must sometimes be brought into a cooler room, in order that the reduction table may be used—and a thermometer is hung over the funnel so that it touches this ; the thermometer is not, however, immersed in the potash.

If bubbles or froth should have accumulated at the meniscus, the rubber tubing leading to the levelling vessel is pinched in the centre with the thumb and forefinger and the rubber tubing leading to the azotometer is hit with sharp taps by the other hand, by which means the potassium hydroxide solution is impelled into the nitrogen and the froth bubbles are broken. Then the cock H_2 of the connecting-piece is opened for the purpose of reading the volume of the gas under the atmospheric pressure at the time.

Reading the Nitrogen Volume

Temperature equilibrium is established fifteen minutes after taking off the azotometer and readings are taken of the temperature to $\pm 0.5°$ C., of the barometer, to ± 1 mm., and of the gas volume, to ± 0.001 c.c. in the azotometer. For this purpose the lens is slid up to the height of the meniscus, the azotometer held by the funnel with the right hand, while the levelling vessel in the left hand is raised until the liquid in it is at the same height as the meniscus, and the reading of the azotometer is taken. Some will find it easier to take the reading whilst the azotometer remains on the bench in the wooden stand. When taking the reading, parallax should be avoided by so adjusting the lens that the scale divisions adjacent to the meniscus coincide with their continuation at the back of the tube. It is necessary to avoid taking hold of the measuring tube or placing it near a lighting unit during the reading, as the consequent warming would lead to an expansion of the gas in a very short time.

It must be emphasised that occasionally after long use the potash solution leaks through the closed cock from the funnel into the upper portion of the measuring tube, owing to deficient lubrication of the azotometer with vaseline. The solution remains at the top and the reading gives too high a result. The potash which has entered can be forced back into the funnel without the slightest loss of gas by raising the levelling vessel above the level of the potash in the funnel and carefully opening the cock. A better proceeding is to press in the cock firmly and to tap down the potash by short strokes on the rubber tubing, as previously described.

Calculation of Results

In consideration of the volume diminution due to the action of the 50 per cent. potassium hydroxide solution, etc., 2 per cent. of the volume v_A of gas read must be subtracted, as previously explained.

The difference is the true nitrogen volume v. The logarithm of the weight of 1 c.c. of nitrogen at the recorded temperature $t°$ and barometric pressure p (millimetres) is obtained from the gas reduction table, No. 7, in Küster's logarithmic tables. From this the percentage of nitrogen for a volume, v, of nitrogen and a weight, s, of the substance is found as follows :

•log (N per cent.) $= \log v + \log$ (factor from table 7) $+ 2 - \log s$.

Example :

2·280 mgm. nitrobenzene ; $p = 712$ mm., $t = 22°$ C.

$$v_A = 0·246 \text{ c.c., } v = 0·241 \text{ c.c.}$$

$C_6H_5NO_2$ { Theory, 11·38 per cent. N.
{ Found, 11·46 per cent. N.

Remarks

The determination of nitrogen according to the process described requires small unimportant changes in exceptional cases only. In the first place, attention should again be drawn to the removal of air before the combustion in the shortest time possible in the case of volatile liquids or solids of high vapour pressure, as recommended previously by F. Pregl. Too low nitrogen values are easily obtained when particular care is not given to the bubbles rising in the azotometer during heating with the movable burner and the froth in the funnel is removed too late. Infusible substances combustible with difficulty must be finely powdered in an agate dish and mixed with a larger amount of fine copper oxide. The mixing of substances which form almost incombustible nitrogenous charcoal with about three times their weight of potassium chlorate gives no unconditional certainty of correct results. This method is accepted for macroanalysis and has also been recommended for microanalysis.

In the analysis of numerous chlorophyll derivatives, which were determined mixed with potassium chlorate and with a mixture of potassium chlorate and potassium dichromate, serviceable values were but rarely obtained. This is due to the fact that the potassium chlorate decomposes even before the non-residual oxidation of the nitrogenous charcoal ; too large an amount of potassium dichromate must be used in order that the substance distributed over a large volume may be completely oxidised.

By slow combustion with strong burners theoretical results may be approached. Serviceable results were first obtained, however, as the entire filling was brought to good red-heat. With the usual roll of wire gauze, the copper oxide filling in the upper part of the tube opposite the flame was not sufficiently heated. The roll was therefore cut through across the middle and a roll of platinum foil of the same length attached to one half. The upper half of the roll formed was, moreover, covered with asbestos paper, in order to prevent loss of

heat as much as possible. For the determination, the roll of wire gauze was fixed over the tube as preheater and the platinum roll heated with a completely non-luminous flame. On account of the high temperature (*cf.* p. 70), the projecting part of the tube was lengthened to 60 mm. In this manner, which probably strains the combustion tube strongly, good results are obtained according to the Dumas-Pregl method.

According to a communication from Dr. E. Wiedemann, Basle, correct nitrogen values can also be obtained in other ways : The combustion is first carried out quite normally until the period of expulsion. The tube is then allowed to cool for about five minutes after shutting off both burners, and the region in which insufficiently burnt residue may be found is carefully tapped. By this the empty spaces formed during the combustion through evolution of gas fall together and the unburnt residue again comes in contact with copper oxide. If this is now ignited for a further time a combustion leaving no residue, with renewed liberation of nitrogen, finally occurs. This method proved excellent for the analysis of chlorophyll derivatives as well as for those of secale, naturally assuming, however, that the micro-bubbles are as small as possible, because otherwise faulty increases in volume may occur in the longer period of the determination.

A. Friedrich [1] prefers the determination according to the micro-Kjeldahl method for substances which are difficult to burn. By this the substances are reduced with hydriodic acid (p. 91) before incineration.

VOLUMETRIC DETERMINATION OF NITROGEN BY F. PREGL'S MICRO-KJELDAHL METHOD

History and Principles

The principle of the Kjeldahl method is : (1) Destruction of the substance with sulphuric acid, whereby the nitrogen is obtained as ammonium sulphate ; (2) steam distillation of the ammonia liberated by alkalies ; and (3) volumetric determination of the ammonia, with N/100 acid. Very small amounts may be determined iodimetrically with N/200 solutions, or colorimetrically with Nessler's reagent.

The first micro-Kjeldahl method was worked out by F. Pilch [2] ; it decomposed the organic substance with 1 c.c. of sulphuric acid, 1 crystal of potassium sulphate, and 1 drop of mercury. F. Pregl [3]

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[1] A. Friedrich : *Z. physiol. Chem.*, **216**, 68 (1933).
[2] F. Pilch : *Monatshefte für Chemie.*, **32**, 21 (1911).
[3] F. Pregl : This book, 2nd (English) edition, p. 110.

used 1 c.c. of sulphuric acid and a mixture of 1 part potassium sulphate and 3 parts copper sulphate for the decomposition. For resistant substances this decomposition may be accelerated by repeated additions of a few drops of Perhydrol (hydrogen peroxide). For albuminous substances F. Pregl recommended addition of mercury.

• According to Pregl's method of incineration, too low nitrogen values are very often obtained. If, however, according to A. Elek and H. Sobotka,[1] glucose is added to these compounds as a reducing agent, the nitrogen is transformed quantitatively into ammonia. The micro-Kjeldahl method thus obtains considerably wider application.

According to A. Friedrich,[2] hydrazine compounds of the carbohydrates yield better values through the addition of glucose than by the earlier decomposition methods, but the results are still not correct. Accurate results were first reached as A. Friedrich carried out the excellent reducing process of decomposition with hydriodic acid. Because this process embraces hydrazines, dinitrohydrazones, osazones, oximes and others as well as nitro-, nitroso- and azo-compounds, and even certain diazocompounds with great accuracy, it is here described fully as the best process at present.

It must, however, be emphasised, that even through the application of hydriodic acid the micro-Kjeldahl method has still not attained that general availability of the micro-Dumas method. It fails, as I have found, completely with diazoketones R—CO—CHN_2 which with hydriodic acid split off almost all the elementary nitrogen even in the cold. The advantage of the micro-Kjeldahl appears on the other hand : (1) In multiple determinations ; (2) in determination of the nitrogen in aqueous solutions (blood, urea, etc.) ; (3) in heterocyclic compounds, which by the micro-Dumas method tend to form nitrogenous charcoals which are difficult to burn.

Apparatus of I. K. Parnas and R. Wagner [3]
(Fig. 26.)

1. The steam generator, A, is a round-bottomed Jena glass flask of 1-litre capacity, which is half-filled with distilled water and acidified with a few drops of sulphuric acid. Pieces of pumice at the bottom prevent delay in boiling and ensure uniform ebullition.

2. The receiver, D, of about 400 c.c. capacity, is connected with the steam generator by a rubber tube. In the neck k is placed a rubber stopper with a hole in it, through which the steam passes to the distillation flask. At the lower end the receiver is constricted. Over the discharge tube, d, is passed a piece of rubber tubing with

[1] A. Elek and H. Sobotka : *J. Amer. Chem. Soc.*, **48**, 501 (1926).
[2] A. Friedrich : *Z. physiol. Chem.*, **216**, 68 (1933).
[3] I. K. Parnas and R. Wagner : *Biochem. Z.*, **125**, 253 (1921).

pinchcock, through which the collected condensed water can be run off at any time.

3. The distilling flask, *B*, is of Jena glass about 30 cm. long, and has an external diameter of about 3 cm. for the upper half and 5 cm. for the lower. This flask forms the actual distillation vessel, which at the top passes into two bulbs *b* and *c*. The sealed-in steam delivery tube, *e*, reaches almost to the bottom of the flask. The distilling chamber is entirely surrounded by a glass jacket *a*, the interior of which is evacuated. Owing to this thermal insulation the time required for the quantitative expulsion of the ammonia is reduced to a few minutes. The steam delivery tube, which is
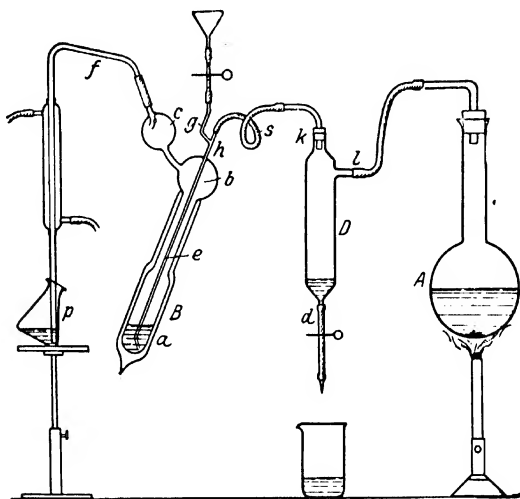
Fig. 26.—Parnas and Wagner's improved micro-Kjeldahl apparatus.

sealed into the foam bulb, *b*, branches into the part, *h*, which is connected by the rubber tube, *S*, with the receiver, *D*. The second ascending part, *g*, is connected with a funnel by a rubber tube provided with a pinchcock. The distilling flask is charged through this funnel. The distilling flask is supported in an oblique position, so that the delivery tube is approximately vertical ; it is connected by means of a rubber tube with the silver condenser tube, *f*, so that the glass and silver are in contact. The portion of the silver tube which connects the two vertical limbs ascends obliquely from the shorter limb, connected with the foam-trap bulb to the longer limb, which is surrounded by the cooling jacket, so that liquid condensed in the oblique portion flows back, and not into the distillate. A Jena glass flask, *p*, of about 100 c.c. capacity, which has been pre-treated by prolonged steaming, and which contains the standard

acid, is brought under the free end of the condenser tube. The use of quartz flasks is desirable, as it renders repeated steaming superfluous.

A quartz condenser tube was found to be quite as good as a silver tube. Owing to its fragility and relatively high cost, however, F. Pregl returned to the silver tube.

The Stand for the Decomposition

For multiple determinations a s t a n d (Fig. 27) provided with six small burners is used. The decomposition flasks are placed in holes of an " Eternit " plate over the burners, with their necks in a suction device, which is connected with the water-pump. The suction device is very useful, especially in laboratories which have poor hoods or none at all.
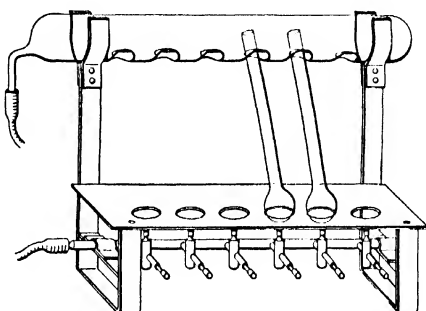
FIG. 27.—Stand for the decomposition, with suction arrangement, connected with the water-pump.

Reagents

Red Phosphorus.
Hydriodic Acid $(d = 1 \cdot 7)$.
Sulphuric Acid $(d = 1 \cdot 84)$.
Mercuric Acetate. A.R.
Potassium Sulphate. A.R.

Thirty per cent. sodium hydroxide solution, which contains 5 per cent. sodium thiosulphate. It is prepared by dissolving 60 gm. of stick sodium hydroxide in 200 gm. of water. To this, 10 gm. of sodium thiosulphate are added. The thiosulphate serves the purpose of decomposing mercury-ammonium-compounds formed by the decomposition of the substance.

N/100 Hydrochloric acid, and

N/100 Sodium Hydroxide solution, which is accurately standardised with methyl red as indicator.

Methyl red (*p*-dimethylaminoazobenzene-*o*-carboxylic acid) saturated solution in N/10 sodium hydroxide.

Course of the Determination

To prevent failure, it is very desirable to ascertain in what form the nitrogen is combined, *e.g.*, whether the substance is volatile below the boiling-point of the hydriodic acid or yields volatile

products split off with hydriodic acid. With certain heterocyclic compounds (antipyrine) and volatile substances the decomposition is carried out in the micro-bomb. For diazo compounds, which easily split off nitrogen as an element, the Kjeldahl method may occasionally be made feasible by coupling with phenol, to form stable azo compounds. For this, the substance is dissolved in three to four times its amount of phenol on the water-bath and treated as usual after cooling. All other substances may be decomposed directly over open flames in Kjeldahl flasks. The Kjeldahl flask of Jena glass is 16 cm. long, with a neck 15 mm. wide which is expanded at the lower end to a bulb of 30 mm. diameter.

Weighing the Substance

Solid substances, which are decomposed in the bomb or in the Kjeldahl flask, are weighed in weighing tubes with long handles, as described in connection with the micro-Dumas method. Solids with considerable electric charges may be pressed into pastilles (p. 233). Viscous substances are weighed in porcelain boats and these are allowed to slide along the wall into the flask. Liquids are weighed in a capillary as in F. Pregl's and J. Pirsch's method, without potassium chlorate. The capillary is slid with the opened tip downwards into the hydriodic acid already in the micro-bomb and is there crushed. If the liquid is very volatile, the bomb must be cooled with ice during sealing; bursting the capillary is omitted.

Physiological liquids (urea, blood, etc.) are brought into the Kjeldahl flask or micro-bomb with Pregl's precision pipette (Fig. 28), which contains a volume of 0·1 c.c. These pipettes are obtainable in different sizes from P. Haack. They are calibrated by weighing with mercury; in the test certificate supplied, is the accurate volume, the weight empty, and also the volume run out up to a millimetre of the capillary; thus making up to the mark is not absolutely necessary. The breadth of the capillary and the tip is so chosen that under the influence of the surface tension and the inner friction the filled pipette may be held vertically without the solution running out. The solution may be estimated with an accuracy of ± 0·0001 c.c. Because the pipette is calibrated for delivery, the residue adhering to the wall must be rinsed again with water from the widened end, or similarly with hydriodic acid for the solution used, in order to remove it.

FIG. 28.— Precision pipette. (½ actual size.)

Incineration by A. Friedrich's Method

Pre-treatment of the Substance in Micro-bomb Tube. One cubic centimetre of hydriodic acid ($d = 1.70$) is allowed to flow down the wall on to the weighed substance and the tube is rotated in order to rinse down particles which remain adhering to it. Capillaries are then introduced into the bomb. As in the Carius determination (p. 106), the bomb is now sealed and heated in the bomb furnace for an hour at 200° C.; for compounds, which contain adjacent nitrogen atoms in a ring, at 300° C. After cooling, the hydriodic acid found in the tip is driven away by gently warming with a non-luminous flame, the bomb is opened and the contents rinsed quantitatively with water into a Kjeldahl flask. The decomposition which follows is carried out in the same way as for substances which have not been pre-treated.

Decomposition. The substance is weighed into the Kjeldahl flask with a few grains of red phosphorus and mixed with 1 c.c. of hydriodic acid from a measuring pipette. For the pre-treated substance, only phosphorus is used. The flask is heated on the stand connected with the water-pump by means of a small flame, till the hydriodic acid boils. After boiling gently for half an hour, the neck of the flask is washed down thoroughly with water, using as much as will half fill the flask. Two cubic centimetres of concentrated sulphuric acid are added from a pipette; then one shakes the mixture and heats it with a strong flame which touches the bottom of the flask, until it boils vigorously and the water and hydriodic acid distil off. After about an hour the iodine and the greatest part of the hydriodic acid have distilled and the solution has become clear. If the neck also is free from sublimed iodine, a small amount of mercuric acetate on the tip of a knife and about two to three times its amount of potassium sulphate are brought into the flask, which is then boiled again for half an hour. The flame is then turned off and the solution diluted carefully with 2–3 c.c. of distilled water.

Steaming. During the decomposition one begins to steam out the distillation apparatus by lighting the burner under the distillation flask; one closes the pinchcocks of the receiver and funnel, and places a collecting vessel under the condenser. Finally, the funnel is steamed by opening the pinchcock and the silver condenser is rinsed outside with distilled water. By the removal of the flame under the steam generator a back pressure is set up, which sucks the whole of the liquid in the distilling flask back into the receiver. The flame is then replaced and fresh steam generated. Finally the pinchcock of the receiver is opened and the collected liquid run off into a beaker.

Application of the Acid. To 8 c.c. of accurately standardised N/100 acid[1] in the 100 c.c. quartz flask, a very small amount of methyl red is added with a glass thread. The quartz flask is brought under the condenser in a somewhat slanting position, so that the end of the condenser tube is well immersed in the acid. Then one tests whether the cold water is running.

Pouring in the Substance. The substance to be analysed is now brought from the Kjeldahl flask through the funnel into the distillation flask. Care must be taken that not a drop is lost. It is advisable to grease the outer rim of the neck of the flask almost imperceptibly, in order to prevent the solution and rinsing water from creeping over. After the substance has been quantitatively transferred to the distilling flask by rinsing four times with a little water, the funnel is finally rinsed out. For rinsing, not more than 10 c.c. of water altogether should be used. To make the solution alkaline, 15 c.c. of 30 per cent. alkali are introduced into the flask through the funnel, from a small measuring cylinder, the funnel is rinsed into the flask with not more than 1 c.c. of water and both pinchcocks then closed.

Distillation. After the pinchcock of the receiver has been closed, steam is at once admitted through the inlet tube to the alkaline solution and heats it rapidly. Thanks to the thermal insulation by the vacuum jacket, only a little water condenses in the distilling flask and the steam soon passes through the foam-bulb to the condenser. All the ammonia is driven into the receiver in three minutes from the entrance of the steam into the condenser ; as a precaution distillation is continued for four minutes. The receiver is then lowered till the end of the condenser tube is about 2 cm. above the acid. Whilst the distillation is continued for another two minutes, the condenser tube is rinsed out at its end with 2–3 c.c. of water and the flask then removed. Before proceeding with the titration the flame is removed from beneath the steam generator.

Titration. The acid solution is boiled up and titrated with N/100 sodium hydroxide from red to a canary-yellow colour, using only a very small amount of indicator in order to obtain a sharp endpoint. The yellow colour should remain for two minutes. The difference between the original acid and that titrated back corresponds to the nitrogen present as ammonia.

Meantime, the apparatus is steamed out as previously described, and after opening the pinchcock of the receiver is ready for the next determination.

Calculation. 1 c.c. N/100 hydrochloric acid corresponds with 0·14 mgm. of nitrogen.

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[1] If necessary, more acid must be taken.

$$\log 14 = 1{\cdot}14638$$

log (percentage of nitrogen) = log (No. of c.c. N/100 HCl used) + log 0·14 + 2 − log (wt. of substance),

where the weight of the substance is expressed in milligrams.

Example :

4·372 mgm. of tyrosine require 2·39 c.c. of N/100 HCl.

Mol. wt. of tyrosine, $C_9H_{11}O_3N = 181{\cdot}1$.

$$\text{Nitrogen} \begin{cases} \text{Calculated} = 7{\cdot}73 \text{ per cent.} \\ \text{Found} \quad = 7{\cdot}65 \text{ per cent.} \end{cases}$$

Remarks

With new apparatus all glass constituents are washed at first with chromic acid in sulphuric acid and with water. The rubber connections are boiled and afterwards well steamed. At the connection of the distillation flask with the condenser by means of pressure tubing, glass and silver must be in close contact ; if the soft moist tubing once becomes dry, there is danger of breaking or leakage when it is connected.

Before the first determination the apparatus is thoroughly steamed out for half an hour, the condensed water sucked back a few times into the receiver, and a blank test made. This gives no nitrogen value with satisfactory reagents and apparatus. If one should be obtained the apparatus and all reagents must be tested. Old tubing is freshly steamed out or replaced by new.

With substances poor in nitrogen, the titration with standard N/100 hydrochloric acid can profitably be made by I. Bang's [1] iodimetric method. It may be assumed that in this case the decomposition of the organic substance should not be carried out with Perhydrol.[2] An unsteamed 100 c.c. Jena flask with ground-in stopper may be used for the iodimetric determination. It is charged with about 5 c.c. of standard N/100 acid without indicator. After the distillation the carbon dioxide is boiled off for three seconds, and after cooling 2 c.c. of 5 per cent. potassium iodide solution and 2 drops of 4 per cent. potassium iodate solution are added to the acid. After standing, closed, for five minutes the liberated iodine is titrated back with N/200 thiosulphate.

The calculation of the nitrogen is similar to that for the acidimetric determination, only the cubic centimetres of N/200 thiosulphate used, corresponding with the acid, are divided by two before subtracting from the standard acid.

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[1] I. Bang : " Methods for Microdetermination of Constituents of Blood," J. F. Bergmann, 1916.

[2] *Cf.* A. Friedrich : " Practice of Quantitative Organic Microanalysis," p. 64, 1933.

III. DETERMINATION OF THE HALOGENS
History and Principles

Various excellent methods for the determination of chlorine and bromine now exist. Among these, Pregl's combustion in bead or worm tubes takes the lead. Although this method requires certain alterations, by the experimentalist, in the preparation of reagents and in experimental details, it is still the method most generally used. It not only permits the analysis of substances of all states of aggregation, but also makes it possible to determine metals (gold, platinum, and many others) by weighing the boat and residue.

Very accurate results are likewise to be obtained by the micro-Carius method, which very rarely fails. It is particularly suitable for multiple determinations and also gives accurate results for liquids.

In the determination of chlorine and bromine by the method of K. J. Zacherl and H. G. Krainick, the organic substance is oxidised by chromic acid in the wet way and chlorine and bromine are determined volumetrically. This simple method, which has proved excellent for a long time in the Kaiser-Wilhelm-Institut, cannot be used for the quantitative oxidation of volatile substances and those of low boiling-point ; iodine cannot be determined by it.

To determine iodine in organic compounds by the gravimetric methods of F. Pregl or Carius is liable to uncertainty in that the precipitate of silver iodide weighed corresponds to only about twice the weight of the iodine present. This process was therefore rightly counted among the most severe micromethods for a long time. T. Leipert has now worked out a very accurate volumetric method by which iodine is titrated as iodate. To-day this method is probably preferred as a rule.

Determination of Chlorine and Bromine by F. Pregl's Method

Because F. Pregl could not obtain correct results with chlormethylanthrone in the bomb-tube at 300° C., he worked out the following process. The organic substance is burnt in a bead tube in a stream of oxygen. The products of combustion pass over red-hot platinum contacts and are absorbed in sodium carbonate solution containing sodium bisulphite. The sodium bisulphite serves the purpose of reducing any halogenate or hypohalogenate. The silver halide precipitated by adding silver nitrate and concentrated nitric acid is weighed.

The method originally devised by F. Pregl for the determination of iodine is now only used for chlorine and bromine.

Worm or Bead Tubes
(Fig. 29).

The decomposition of the organic substance takes place in a combustion tube of Jena glass, 500 (or, better, 600) mm. long. At

one end the tube is drawn out to a thick-walled tip, the bore of which should be ½ mm. The fine opening only allows the washings after the completion of the combustion to run through slowly, and thus lengthens the time of contact with the beads or the worm, so that the beads may be rinsed quantitatively with small amounts of liquid. In front of the point there are two depressions in the tube, which prevent a bead from lodging at this point on washing out. The adjacent portion of the tube is filled to a length of 200 mm. with Jena glass beads *Ps*, of about 3·5 mm. diameter, or with a glass spiral.[1] To prevent the beads from falling out, the tube is provided with two more parallel depressions behind these, which reduce the cross-section just sufficiently for this purpose. If the cross-section at this point is too narrow, it becomes difficult to fill that portion of the tube containing the beads with water without an air space.

The Platinum Contacts

The platinum contacts made by C. Heraeus Hanau consist of platinum cylinders 70 mm.
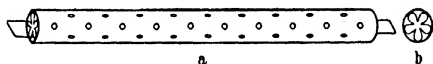
Fig. 30.—(a). Platinum contacts from two perforated cylinders which slide into one another and welded on platinum loops. (b). Cross-section of the platinum cylinder. (⅔ actual size.)

long, which are perforated in places. Their diameters are about 7 mm.; they should only be smaller than the bore of the combustion tube by a few tenths of a millimetre. In order to remove the cylinders after the combustion, small loops of platinum are welded on at both ends of the strengthened
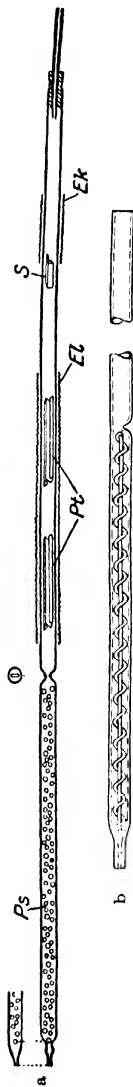
Fig. 29.—(a). Bead tube. (b). Spiral tube for determination of halogens and sulphur. (⅓ actual size.) *Ps.* Hard glass beads. *Pt.* Platinum contacts. *El.* Long roll of iron gauze. *Ek.* Short roll of iron gauze. *S.* Boat.

[1] Bead tubes may be obtained from P. Haack, and, according to A. Friedrich, spiral tubes also. At the Kaiser-Wilhelm-Institut and elsewhere, very good experience was obtained with spiral tubes for determination of halogens and sulphur. The first tubes contained porcelain beads, which occasionally were porous or became cracked in course of time, and in spite of careful rinsing retained the products of combustion in their cracks, which led to high results. A. Soltys replaced the beads by a glass spiral tube which he had made himself. Even in the first determinations the filling was shown to be superior to the beads.

cylinder edges. In the outer cylinder are placed the platinum stars, which in cross-section have the shape of a five-leaved clover. If the products of combustion, mixed with oxygen, are streamed slowly through both contacts, volatile substances are completely oxidised.

According to F. Pregl, these contacts are made thus :

Platinum foil (0·05 mm. thick), 50 mm. long and 15–18 mm. broad, is so bent together longitudinally that the cross-section resembles a Latin " Z " with somewhat lengthened initial and final strokes. For this purpose two parallel lines, 5 mm. apart, are drawn on a rather large sheet of paper, and the platinum foil is placed longitudinally over this, so that its centre is half-way between the two lines and so that it extends equally on either side. Points are also marked that bisect the distances between the parallel lines and the edges of the platinum strip. At these points and lines the foil is bent longitudinally by pressing on to it a piece of pasteboard with a straight edge, and bending the projecting portion over. When the bends have once been commenced, they are easily completed by hand until the whole contact, which is 50 mm. long, has throughout the above-mentioned cross-section of a Latin " Z." In spite of the small thickness of the material it offers considerable resistance to distortion and bending. These contacts are easily pushed into the combustion tube. They may easily be ·removed with the help of a hook of platinum wire, sealed into a long glass rod, particularly if the ends of the contact are slightly bent over, or if the edges at the narrow end of the foil are provided with perforations at one or two places, into which a hook can be inserted.

Both platinum contacts must be boiled in dilute nitric acid and fully ignited in a flame before use, and are then at once brought into the bead-tube. It may happen that the contacts lose their catalytic activity through " poisoning " ; this may be restored by etching them with hot aqua-regia.

The contacts should always be kept in a glass dish covered with a clock glass, and should not be handled except with a platinum-tipped forceps.

The Filter-tubes

Until 1912 F. Pregl used a micro-Gooch crucible for filtering the precipitates of silver halide ; this was soon replaced by the Neubauer crucible. A wish to convey the silver halide precipitate automatically on to the filtering surface led to the construction of the filter tube.

The first filter tubes had a constriction at the junction of the stem, on which a spiral platinum wire was placed, which supported the asbestos filling. On rinsing the tube, leakage was sometimes caused by swelling of the asbestos. With filter tubes which possess

a cavity for the asbestos pad (Fig. 31 (*a*)), swelling and a creeping
through of the silver halide precipitate may be avoided. However,
on account of the large asbestos pad the greatest attention had to
be paid to the current of air during the separate dryings, in order
that trouble should not be caused by incomplete
drying of the filter-tubes.

• To-day the Jena filter-tubes are principally
used. The required density of the filter for a
constant velocity of filtration is easily attained
through a thin layer of asbestos consolidated by
suction on the fritted glass mass.

The total length of the filter-tube is 15 mm.
The upper 4·5 c.c. consist of a glass tube 1 mm.
bore for the admission of the solution to be
filtered. A fritted glass mass (154 G 1) 2–3 mm.
thick closes up this part at the bottom. About
1 cm. below the filter-layer the tube is conically
constricted and passes into a shaft about 10 cm.
long and 3 mm. bore.

Reference may here be made to F. Emich's [1]
method of working with a suction rod, which
permits of the collection and washing of the most
varied precipitates. This process has already
been used successfully for widely different quan-
titative determinations on organic substances.
It is certainly necessary to adjust the process
next described to the previous process in some
details. [2]

The preparation of the filter-tube is carried
out in a suction apparatus (Fig. 32).

a.   *b.*

Fig. 31. — Filter
tubes for preci-
pitates of silver
halides and phos-
phoammonium
molybdate.
(*a*) Old form. (*b*)
New fritted glass
tubes. (⅔ actual
size.)

The Suction Apparatus

(Fig. 32)

This consists of a 250 c.c. wide-necked filter-
flask. In the neck is placed a well-fitting rubber
stopper, with a glass tube, 8 cm. long and of 8 mm. bore, passing
through a hole in it. On the upper end of this tube is a piece of
rubber tubing, 20 mm. long, which should project 10 mm. above
the edge. The filter-tube passes through a small rubber stopper in
this collar.

The silver halide precipitates are drawn into the filter-tube by
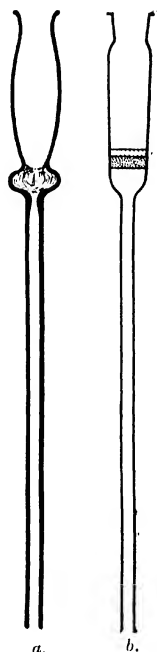means of a syphon. The syphon can best be made from a thin glass

[1] F. Emich : " Lehrbuch der Mikrochemie," 2nd edition, pp. 84–88, 1926.
[2] *Z. analyt. Chem.*, **71**, 117 (1927).

tube, 3 mm. bore, over a small fish-tailed burner. The long vertical part is 20–25 cm. long and at the top is bent through an angle of rather more than 90°, then bent again, after 8–10 cm., parallel to the first tube. The last part is about 6 cm. long.

The **Filter-tube** is next placed in the moistened rubber collar of the filtering flask. A suspension of medium-fine asbestos, 2 mm. thick, is obtained on the fritted glass under gentle suction. This is uniformly distributed and pressed down with a sharp-edged glass rod. This operation is repeated twice more, using a very fine suspension, until the compressed asbestos layer is 1·5–2 mm. thick, with a flat surface. It is then washed well with water, three times with hot sulphuric-chromic acid, and again with water. Finally, hôt nitric acid, water and alcohol are aspirated through. The filter-tube is dried in a regenerating block at 120° C. whilst air is simultaneously aspirated through it. (On drying, see p. 102.)

Fig. 32.—Suction apparatus for determination of halogens and phosphorus. (⅓ actual size.)

R. Wide test-tube. *H.* Syphon. *F.* Filter-tube. *S.* Rubber stopper. *Gm.* Rubber collar. *Gr.* Adjustable glass tube.

The Drying Block, or Regenerating Block

This consists essentially of two superposed copper blocks (Fig. 33), each of which is provided with two semicircular channels, which together form cylindrical canals. One of these channels has a diameter of 12 mm. and serves to hold the widened portion of the filter-tube. The second is narrower and has a diameter of 8 mm. The two copper blocks are heated with a micro-burner. The temperature is read on a thermometer, which fits horizontally into a socket provided for it. The micro-burner is provided with a delicate regulating screw, which regulates the temperature constant to 2°–3° C.

Fig. 33.—The drying block, or regenerating block. (⅓ actual size.)

Reagents

Obviously all reagents used must be absolutely free from halogen.

Distilled Water. When 10 c.c. are mixed with 5 drops of nitric acid and the same quantity of silver nitrate solution and warmed for ten minutes on the boiling water-bath, no trace of opalescence should be shown. It is advisable to close the mouth of the stock bottle with a soda-lime tube.

Concentrated Nitric Acid. If this should contain chloride it is purified by distillation *in vacuo* over silver nitrate and kept in a brown bottle with a ground-glass stopper and glass cap.

Sodium Carbonate Solution, free from Halogen. This is prepared by B. Reinitzer's method.[1] About 500 gm. of commercial sodium carbonate are stirred up with a little water to a paste; this is drained on to a Buchner funnel, and the operation repeated until the last filtrates show scarcely any chloride reaction. The washed sodium bicarbonate[2] is added with stirring to about 2,000 gm. of water at 80° C. in a Jena glass beaker until a portion remains undissolved.* After each addition there is a strong evolution of carbon dioxide. The hot solution is then filtered through a folding filter, which has been previously washed with distilled water free from halogen, and the filtrate cooled in a stream of water. After standing for some time a salt crystallises out, of the composition $Na_2CO_3 + NaHCO_3 + H_2O$. After draining on a Buchner funnel and washing with a little water, the salt is tested by suspending 1 gm. in water, adding nitric acid and silver nitrate, and heating for five minutes on the boiling water-bath. If the operation has been carried out successfully, not the slightest trace of opalescence should be shown after cooling. The purified salt is carefully dried and stored in well-stoppered wide-necked bottles, which are further protected by parchment as a precaution, in readiness for the preparation of the solution. This salt is added to boiling distilled water in such quantity (20-25 gm. per 100 c.c.) that a saturated solution is obtained. This is poured into the stock bottle while still warm; sodium bicarbonate crystallises out on cooling. Jena glass bottles, with rubber stoppers, are now used for storing the salt, as ordinary bottles are attacked. If the solution has been used up, a further quantity can be obtained by adding distilled water to the sediment.

Bisulphite Solution free from Halogen. This is prepared from the concentrated sodium carbonate solution free from halogen by slowly introducing sulphur dioxide free from halogen, with cooling. Should the temperature rise during the introduction of sulphur

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[1] *Z. analyt. Chem.*, **34**, 574 (1895).
[2] Sodium bicarbonate (p.a.) prepared by E. Merck does not require this purification.

dioxide, appreciable quantities of thiosulphate are often formed, which give rise to the separation of sulphur on acidifying. The sulphur dioxide is prepared from concentrated sodium bisulphite solution by slowly adding concentrated sulphuric acid (A.R.) and passing the liberated gas through a tube containing glass wool moistened with concentrated sodium carbonate solution free from halogen, before it is introduced into the cooled sodium carbonate solution.

Test-tubes are half filled with the sodium bisulphite solution and then sealed so that a long capillary is produced. A considerable quantity of pure solution can be stored in this way. Before use the drawn-out point of the capillary is opened and the solution is expelled, drop by drop, by warming with the hand. The point is then sealed.

The sodium bisulphite solution is tested as follows : Twenty to forty cubic centimetres are made alkaline with sodium carbonate solution free from halogen and warmed on a water-bath with 3 to 5 drops of perhydrol for five minutes. After cooling,·a mixture of 1–2 c.c. of nitric acid free from halogen and 0·5 c.c. of silver nitrate solution is added and the liquid is heated for ten minutes on the boiling water-bath. No trace of turbidity should be produced.

Perhydrol. Guaranteed free from acid.

Silver Nitrate Solution. A 5 per cent. solution is prepared from 5 gm. of silver nitrate in 100 c.c. of water. Any turbidity settles overnight and does not affect its suitability.

Wash Bottles. Two wash bottles, with ground glass joints, of about 250 c.c. capacity are required. One is filled with 96 per cent. alcohol, the other with distilled water to which a little nitric acid (1 : 200) is added.

Course of the Determination

A wide Jena glass test-tube and the combustion tube are carefully cleaned with warm sulphuric-chromic acid and water. The tube is cleaned by first closing the neck with rubber tubing by means of a pinchcock, and pouring into the wide opening first a little sulphuric-chromic acid, till the air is driven out of the spiral, then filling up and placing in a stand. After fifteen minutes the sulphuric-chromic acid is run off and the tube washed well with distilled water followed by alcohol or acetone, and dried with the neck connected to the water-pump. For drying, the mouth of the tube must be provided with an air filter (Fig. 34). By carefully warming the tube over the flame, drying may be accelerated.

The substances to be analysed are weighed in platinum boats as in the carbon-hydrogen determination, using somewhat larger amounts (4–8 mgm.) of substance. If liquids are burnt in capillaries,

obviously not potassium chlorate but ammonium nitrate should be used as propellant.

To the cleaned test-tube previously mentioned, 2 c.c. of the saturated sodium carbonate solution and 3 drops of the bisulphite solution are added. This mixture is aspirated slowly into that portion of the combustion tube containing the beads or spiral, to a height such that the liquid moistens all the beads ; the liquid must not be drawn higher. The solution is now allowed to flow back into the test-tube, which it easily does if no air has been drawn into it ; the remainder is then carefully blown out. After pouring away the solution the test-tube is placed over the neck of the combustion tube and remains there till the end of the combustion. The combustion tube is now so placed on the combustion stand that the spiral or bead layer and 4 cm. of the empty portion which adjoins it project beyond the combustion stand. This projecting part, which can be seriously distorted during heating, is protected by a micro-stand. The platinum contacts, which have been boiled in nitric acid and ignited, are placed in the tube by means of a platinum-tipped forceps and pushed over the tube burner with a glass rod. In order to prevent iron particles from dropping into the tube later, its mouth is closed with a cork. The longer and shorter wire gauze rolls are then passed over the tube, the long one over the tube burner and the short one about 7 cm. further in front. The stopper is removed and the boat or the capillary with broken tip is introduced to a distance of about 5 cm. from the tube burner. The wire gauze tunnels are brought over the rolls of wire gauze. The tube is now closed by a perforated rubber stopper, into which a glass capillary for the introduction of oxygen fits well. The oxygen is preferably supplied from a gas-holder directly through a reducing (needle) valve. Between the bomb and the combustion tube is a wash-bottle containing a saturated solution of sodium carbonate. If the oxygen is supplied from gasholders, it is convenient to introduce into the rubber tubing between the wash-bottle and the combustion tube a little twisted cotton-wool or a few lengths of string, in order to facilitate regulation of the bubble frequency by a pinchcock so that 4 c.c. of oxygen per minute pass across the tube.

The speed of the current of oxygen from the bomb makes the adjustment of this bubble-frequency possible without difficulty by means of a needle valve ; a pinchcock is superfluous.

The platinum star contacts are first heated slowly to redness with the tube burner ; then the combustion of the substance is begun. For this, the short roll of wire gauze is brought up to 1 cm. of the boat and heated in the middle with the non-luminous flame of the movable burner. The combustion is carried out with all the precautions previously described for the determination of carbon

and hydrogen. The combustion must, however, be carried out three times as slowly, and takes half an hour. This is due, on the one hand, to the less active platinum contacts, and, on the other, to the slower absorption through the sodium carbonate solution. It must not be forgotten that no choking plug is present, and therefore substances which are heated too quickly easily pass partially unburnt into the sodium carbonate solution. When the movable burner is advanced up to the tube burner, heating is continued two minutes longer, then both burners are extinguished and the tube allowed to cool in a current of oxygen.

Meanwhile the regenerating block is heated to 120° C. and the empty filter-tube, prepared as on p. 98, placed with the stem in a moistened rubber stopper in the collar of the filtering apparatus. The vacuum is adjusted by means of pinchcocks,[1] then a gentle current of air is drawn through and the filter-tubes are washed twice with water acidified with a little nitric acid and finally with alcohol. The mouth of the filter-tube is closed with an air filter, to avoid increase in weight caused by the dust particles from the air of the laboratory. The air filter (Fig. 34) consists of a funnel filled with cotton-wool, which is placed in a cork in the filter-tube. After washing, the filter-tube is taken from the rubber stopper, wiped on the outside with a towel, and the stem placed in a tight-fitting rubber tube, connected with the vacuum pressure tubing by a connecting piece. The tube is dried at 120° C. for five minutes in the regenerating block while air is drawn through as before. Finally, the stem is placed in the narrow hole to remove the last traces of moisture. The dried filter-tube is taken from the pump and wiped after removal of the air filter. The tube must always be placed mouth upwards, because the silver halide precipitate does not always adhere to the asbestos. It is wiped first with moist flannel and then with chamois leather, as described for the absorption tubes (p. 43), then placed on the stand next to the balance and allowed to cool for fifteen minutes. It is placed on the balance with the fork and weighed accurately to 0·005 mgm. five minutes later. New filter-tubes are counterpoised against flasks containing shot (p. 14). Filter-tubes may be used for the consecutive determinations of alkoxyl groups by weighing silver iodide, as well as for precipitates of silver chloride and bromide. If 60–80 mgm. of silver halide have been collected a slackening of the velocity of filtration becomes noticeable, and it is advisable to dissolve the precipitate by means of warm potassium cyanide solution and to clean the tube as before with water, chromic acid, water containing a little nitric acid, and alcohol.

Fig. 34.— Air filter with cork.

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[1] A cock with long spindle, as in the micro-Dumas method, may be used with advantage.

The boat and the platinum star contacts are withdrawn from the cooled combustion tube with a platinum wire hook, after removal of the stopper containing the capillary. If a residue is visible in the boat by means of a lens, the weight of this must be determined. Before the roll of wire gauze is removed, a cork is inserted again, as above, in the mouth of the tube. Particles of iron from the roll are carefully removed from the tube with a cloth. Whilst the test-tube is held over the neck of the tube, this is in a stand clamped at an acute angle with the vertical so that the test-tube stands upon the bench and the neck of the combustion tube in the test-tube is about 4 cm. high. The cork is now removed, 2–3 drops of bisulphite solution brought into the tube, and the beads or the spiral tubes are completely covered with water in a continuous stream from the wash-bottle. With bead-tubes, air-locks sometimes prevent complete covering ; on rotating the tubes, air-bubbles escape. After running off the first washings, the tube is rinsed twice more with the same amount of acidified water, and finally the neck is rinsed.

The collected washings are treated with 2 drops of perhydrol to oxidise the sulphite, and are then heated in a boiling water-bath for five minutes, after inverting a beaker over the test-tube to protect it.

A mixture of 1 c.c. of concentrated nitric acid and 2 c.c. of silver nitrate solution is added to the hot solution, when an opalescent cloudiness is first obtained. After heating again for ten to fifteen minutes in the boiling water-bath, the precipitation is quantitative, and the silver halide, which has clotted together, may be filtered after cooling the solution.

The weighed filter-tube is placed in the filter-flask. The syphon, which is cleaned before each series of determinations with warm sulphuric-chromic acid, water and alcohol, is placed in a rubber stopper which exactly fits in the mouth of the tube so that the syphon ends 2 cm. below the stopper. The stopper is moistened with distilled water to obtain a completely air-tight connection. The filtered solution drops on to the asbestos without touching the wall of the filter-tube. The long limb of the syphon is lowered till just above the precipitate in the test-tube and the pump turned on carefully till about 2 drops of filtrate pass per second. After the bulk of the liquid has been removed, the inner surface of the test-tube is rinsed with the acidified water as before, shaken well, in order to whirl the precipitate, and the whole of the precipitate is then syphoned off. If particles should still adhere to the walls, they are rinsed to the bottom of the test-tube, as much as possible, with a fine stream from the wash-bottle. In order to transfer the last particles of silver halide to the filter, the walls of the test-tube are finally rinsed with alcohol and these traces are thereby removed through the surface tension phenomena at the junction of alcohol and water. The alternate rinsing with acidified water and alcohol is repeated twice more. It is only necessary to

loosen adherent particles of the precipitate with the feather used in the determination of sulphur, which will be described later, in exceptional cases.

When filtration is finished the rubber stopper with the syphon is carefully removed from the filter-tube and the portion of the syphon within the tube rinsed with alcohol, and the filter-tube filled with alcohol up to the brim. After the alcohol has run through, the air filter is put on. The drying and weighing of the tube and precipitate are carried out exactly as described on p. 102.

Calculation :—1 mgm. Cl corresponds with 4·043 mgm. AgCl.

1 mgm. Br corresponds with 2·348 mgm. AgBr.

log (percentage of halogen) = log (mgm. of silver halide)
    + log (factor) + 2 − log (mgm. of substance)

	Factor	Log (factor)
$\dfrac{Cl}{AgCl}$ . .	0·2474	$\bar{1}$·39334
$\dfrac{Br}{AgBr}$ . .	0·4255	$\bar{1}$·62894

Example : 3·615 mgm. chlorobenzoic acid yield

3·33 mgm. AgCl.

Mol. wt. of acid, $C_7H_5O_2Cl = 156·5$

Theory, 22·66 per cent. Cl.

Found, 22·79 per cent. Cl.

Determination of Chlorine and Bromine by Carius' Method

The first micro-determinations of the halogens and sulphur were carried out by F. Emich and J. Donan.[1] The Nernst balance was used, to the limitations of which the objects to be weighed had to be adapted. The form of this method which follows was developed by F. Pregl in 1910 ; it is advisable for series of analyses. The Kaiser-Wilhelm-Institut has also found it excellent for this purpose ; it requires very little time and is not, as a rule, inferior in accuracy to the combustion in the tube filled with beads. With hydroquinone-di-(ω-brom)-hexylether, however, in spite of heating the bomb for up to seven hours at 300° C., I was often only able to obtain uncertain results which were much too low, while the combustion in the bead-tube at once gave correct values. Although the method fails in individual cases, it is nevertheless very valuable ; it is easily manipulated by inexperienced microanalysts.

The Small Pressure Tube

Hard Jena glass, 20, is most suitable for making pressure tubes. They should be 10–12 mm. internal diameter and 1–1·3 mm. thick ; so that they may be used more often, they are 250 mm. long.

[1] *Monatshefte f. Chem.*, **30**, 745 (1909).

Before use, they are filled with sulphuric-chromic acid and placed in a high beaker in a boiling water-bath for fifteen minutes, washed with distilled water, and dried at 115° C. in an oven. The thorough drying is important in view of the technique of the weighing of the substance.

The Tube Furnace

* The tubes can be heated in any tube furnace. According to statements by F. Pregl, one made by P. Haack is to be highly recommended. This is of a size very suitable for the dimensions of the pressure tubes. It has the advantage that the required temperature can rapidly be attained and also the furnace can be quickly cooled again. In order that the temperature may easily be controlled, it is preferably placed in the hood of the micro-laboratory and a strong board is placed before the small suspension doors as a protection. With electrically heated furnaces [1] it is very advisable to insert a clock-mechanism between the relay and the furnace, which automatically cuts off the current after five hours.[2] Pressure tubes can thus be placed in the furnace in the evening and be found cooled down next morning.

Course of the Determination

For weighing, F. Pregl recommends that *solid substances* should be pressed into a hard glass capillary open at both ends and the capillary weighed in a horizontal position on a stand of aluminium wire. I gave up this type of weighing four years ago, in order that the precipitate of silver halide might be syphoned from the pressure tube without delay. For this purpose, all solids (3–8 mgm.) are inserted as deeply as possible into the pressure tubes by means of the weighing tubes with long handles (see p. 79) ; particles adhering to the wall are tapped to the bottom with a wooden rod. Only substances which evolve halogen even in the cold on contact with nitric acid are to be weighed, according to A. Friedrich's [3] method, in a hard glass tube, 15 mm. long and 3 mm. bore, sealed on one side. After charging the pressure tube with silver nitrate and nitric acid, the pressure tube is pushed down the wall to the bottom of it.

The micro-weighing bottles, which can easily be prepared from hard glass tubes, are particularly suitable for weighing liquids.[4] If a ground-in stopper is used, even substances with slight vapour pressure may be weighed.

If the liquids are *volatile*, they must be weighed by J. Pirsch's method (p. 242) ; the handle of the capillary should here be a glass

[1] W. C. Heraeus.
[2] Obtainable from A. Huber, Munich.
[3] A. Friedrich : " Practice of Quantitative Organic Microanalysis," p. 86.
[4] It is also used for weighing liquids in the determination of the alkoxyl, acetyl, and isopropylidene groups and in the determination of active hydrogen.

rod, 10–15 mm. long, 3 mm. thick, and proportionately heavier. The capillary filled with the substance and with the hair capillary underneath is pushed to the bottom of the charged pressure tube and the tube immediately sealed. To burst the capillary the cooled tube is held point upwards in one hand and quickly hit from below a few times with the other hand, thus bursting the capillary by means of the heavy glass rod. It is obvious that the precipitate which is mixed with fine splinters of glass, must be weighed in a freshly prepared filter-tube which must be weighed again after dissolving the silver halide with potassium cyanide solution. With volatile liquids, careful combustion in the bead-tube is preferable.

Oils and *liquids of a syrupy consistency* are introduced into the boat with a thin glass rod or weighed in the small weighing-bottle (Fig. 35).

To the

Fɪɢ. 35.— Micro-weigh-ing bottles, with and without ground-in stoppers. (Actual size.)

substance, one or two crystals of silver nitrate (10–20 mgm.) are added and 0·5–0·8 c.c. of halogen-free concentrated nitric acid are introduced by a fine pipette, the tip of which passes about 5 cm. into the slanting pressure tube. The nitric acid is allowed to flow slowly down the side of the tube whilst this is rotated, to rinse down the last particles.

A thick glass rod and the open end of the charged pressure tube are pre-heated in the luminous blowpipe flame ; the glass is then sealed to the inner edge of the tube in the non-luminous flame. For this the tube is heated in the hottest part of the flame, 2–3 cm. below its mouth, whilst rotating slowly till the glass begins to fuse and flow together ; it must not be drawn out. When the walls of the tube finally close together to a capillary, the point of fusion is drawn out to a thin-walled capillary in the outer part of the flame, and sealed together. To avoid straining the glass the sealed end is cooled in a smoky flame.

Organic substances are usually decomposed by heating at 280° C. for five hours ; 300° C. is better for halogens in the benzene ring.

After cooling to room temperature, the tube, in its slanting position, is partially withdrawn from the oven by a length of about 50 mm. Before opening, the nitric acid is driven from the capillary and the upper part by touching these frequently with the just non-luminous flame of a Bunsen burner. The point is then softened with the non-luminous flame until it opens under the slight inside pressure. A scratch 1 cm. long is made in the opened tube with a glass-cutter, at 1 cm. below the constriction, and the scratch broken with a red-hot drop of glass. To prevent particles of glass from falling into the nitric acid, the tube is held almost horizontal and the broken point carefully removed, the sharp edge rounded in the

blowpipe flame and the tube softened to a distance of 2–3 cm. down, to absorb any adherent splinters. This precaution is most essential, because too high halogen values are almost always due to glass splinters in the precipitate.

J. Unterzaucher's Method of Opening the Pressure Tube [1]

Dr. J. Unterzaucher has kindly sent me the following communication on this process :

"The danger of contamination of the precipitate with glass splinters, which is always present in bursting open Carius tubes, may be completely avoided by a new process discovered by my former laboratory assistant P. Rös-cheisen. This consists of melting open and cutting the Carius tubes by an oxygen-illuminating gas flame, and is carried out as follows :

"The cooled Carius tubes, which are still in the furnace, are next opened as usual by softening the extreme end of the capillary by means of strong Bunsen flames, in order to blow off the pressure, and are afterwards closed again by sealing in the same place with the oxygen-illuminating gas flame. The blowpipe is now adjusted ; a fine-pointed oxygen flame, 2–3 cm. long, is produced by leading oxygen at the required rate into the gas flame. The Carius tube is now warmed in

Fig. 36.—Splinter-free opening of the pressure tube, by J. Unterzaucher and P. Röscheisen.

the flame at 3–4 cm. from the bellows whilst constantly rotating, thus avoiding contact with the flame. After pre-heating sufficiently, the tube is brought tangentially in contact with the blue cone of the oxygen flame. After considerable rotation, by which the tube may be taken somewhat further into the oxygen flame, a ring-shaped softening of the glass occurs. Rotation is now stopped and part of the ring heated directly with the fine-pointed flame, till the inside pressure developed by heating bursts a small oval opening in the wall of the tube, as may be seen from the diagram in Fig. 36 (a, front view ; a^1, side view).

"The oxy-flame is directed now on the side end of the oval opening and the tube rotated whilst simultaneously bending it
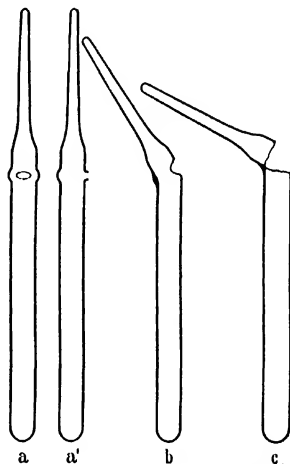
[1] Unpublished,

backwards to such an extent as the softening and tearing of the glass will permit (*b*), so that the sideways widening of the oval opening forms a cut around the whole tube (*c*).

" Bursting of the tube, therefore, need not be feared; however, the use of dark spectacles is urgently demanded because of the brilliant light of the oxygenated flame.

" The process also possesses the advantage of being easily carried out, with corresponding saving in time."

The sides of the tube are rinsed down with 2 c.c. of acidified water whilst being rotated. With much silver halide, the precipitate which collects into a ball may enclose silver nitrate, which leads to false results. To avoid such an error, the balled precipitate is crushed in the acid with a clean rounded-off glass rod and rinsed in the tube with acidified water. If the substance is to be weighed in the porcelain boat or in a weighing-bottle, it is drawn to the mouth of the tube with the platinum hook, seized with platinum-tipped forceps and rinsed thoroughly into the tube ; the nitric acid is diluted to about 3 c.c. with water, and the tube placed on the boiling water-bath for about five minutes in a high litre beaker. Immediately the solution is cooled it is syphoned directly on to the filter-tube as previously described (p. 98).

Calculation. As on p. 104.

Finally, it must be stated that the opened pressure tube should not remain for days in the light, because this usually leads to precipitation of silver and to results which are too high. With series of analyses it is therefore advisable to filter the precipitates immediately in succession.

Alkalimetric Determination of Chlorine and Bromine by the Method of M. K. Zacherl and H. G. Krainick [1]

On the basis of the method of H. Baubigny and G. Chavanne,[2] the organic material is oxidised, in a current of oxygen, with concentrated sulphuric acid in the presence of potassium dichromate and silver dichromate. Chlorine and bromine are thereby volatilised, whilst iodine is quantitatively retained as iodate. The halogens are carried over by the oxygen into an absorption apparatus, where they react with a measured amount of sodium hydroxide solution and perhydrol,[3] according to the equation :

$$Cl_2 + 2NaOH + H_2O_2 = 2NaCl + 2H_2O + O_2.$$

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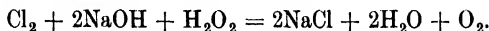
[1] *Mikrochem.*, **11**, 61 (1932).

[2] *C. R. Acad. Sci. Paris*, **136**, 1197 (1903). S. Nomura and J. Murai, *Bull. Soc. Chim. Fr.*, **35**, 217 (1924) and H. Dieterle, *Arch. Pharmaz.*, **261**, 73 (1925) have modified the macro-method to one for micro-analyses, while keeping the gravimetric basis.

[3] *Arch. Pharmaz.*, **262**, 3 (1924).

The unused alkali is titrated back, using methyl red as indicator, and a blank test on the acidity of the perhydrol is made.

Apparatus

This is made of Jena glass, and consists of the oxidation vessel with ground-in attachment (Fig. 37).

• The oxidation vessel is 100 mm. long and has a neck 80 mm. long and of 10 mm. inner diameter. The bulb is heart-shaped, and 18 mm. in diameter at the widest part. The ground-in attachment is held fast in the neck, to a depth of 20 mm., by means of hooks and steel springs. Through the stopper there passes an inlet tube of 2 mm. bore, 90 mm. long, and ending 4 mm. from the bottom of the oxidation flask. Above the joint the inlet tube is connected with the dropping funnel T by a stop-cock H_1. This funnel has a capacity of 4–5 c.c., and has a ground-in inlet tube, of 8 mm. bore and bent at right angles, at the top. This is similarly held by steel springs. The delivery tube to the absorption apparatus branches off immediately above the joint of the flask. After 5 cm. it is bent downwards at right angles and slightly constricted at the end. The vertical tube is about 27 cm. long and 2 mm. inner diameter.

The absorption apparatus consists of a glass tube of 12 mm. diameter, which widens into a funnel at the top. At the lower end there is a well-fitting stop-cock H_2, and the tube ends in a thick-walled outlet 4 cm. long and 1–2 mm. bore. The central inlet tube is closely surrounded by a massive spiral of Jena

Fig. 37.—Apparatus for the determination of chlorine and bromine by the method of M. K. Zacherl and H. G. Krainick.

Ground-in attachment S with dropping-funnel T, closed by H_1. Oxidation vessel O. Absorption apparatus D with closely fitting spiral Sp, of Jena glass.

glass, which reaches the bottom of the funnel and touches the Wall of the outer tube. This compels the bubbles of gas which escape at the tip of the inner tube to rise by a circular motion and to remain in contact with the solution as long as possible.
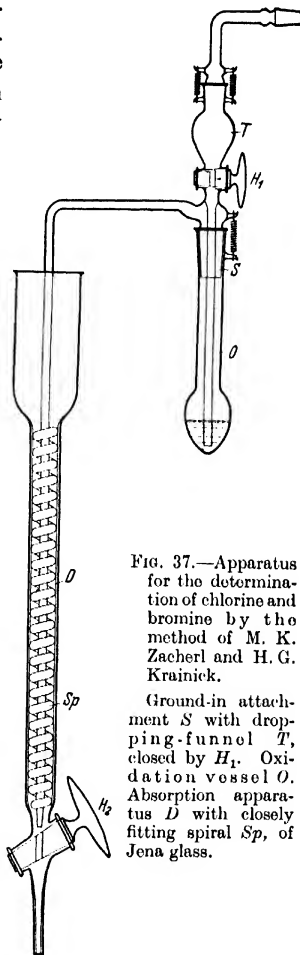
The Aluminium Heating Block. Halogen determinations are usually carried out in the same room as the carbon and hydrogen determinations. It is therefore obvious that organic vapours from baths of oil or paraffin must be avoided as much as possible.
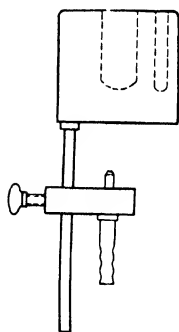

Fig. 38.—Aluminium heating block with micro-burner. (½ actual size.)

The aluminium block with micro-burner (Fig. 38) has been approved for some time and it enables the temperature to be obtained accurately to $\pm$ 2° C. It consists of a solid aluminium cylinder, 80 mm. diameter, 70 mm. high, which is clamped in a stand by means of a screwed-on brass rod. The central cylindrical hole, 24 mm. bore, serves for the admission of the oxidation flask, whilst the thermometer is placed in the narrow, 8 mm., hole. The micro-burner may be screwed fast to the vertical brass rod at any height required. The flame is regulated by means of a pinchcock on the tubing.

Reagents

Concentrated Sulphuric Acid ($d = 1.84$), A.R., 2 c.c. pipette.

A mixture of equal parts by weight of *potassium dichromate*, A.R., and *silver dichromate*. The silver dichromate is best prepared according to the directions of W. Autenrieth [1] as follows :—

Ten grams of silver nitrate and 6 gm. of chromic acid (A.R.) are boiled with 1 litre of distilled water until all is dissolved. After a few hours the silver dichromate begins to separate in dark brown, glittering crystals. It is best to allow it to stand overnight and then to pour off the supernatant liquid. The crystals are washed twice on a Buchner funnel with distilled water and dried over phosphorus pentoxide in a desiccator. The dried and powdered silver dichromate is used for the oxidising mixture, which is stored in a brown wide-necked bottle.

Perhydrol (E. Merck's finest quality, guaranteed free from acid). Because even the " guaranteed free from acid " gives a slightly acid reaction, its acidity must be determined on every new bottle. One cubic centimetre of perhydrol is mixed with 1–2 c.c. of N/100 hydrochloric acid, boiled, and neutralised with N/100 sodium hydroxide solution, using methyl red as indicator.

Standard N/100 *Hydrochloric Acid*, and *Standard* N/100 *Sodium Hydroxide Solution*, in automatic micro-burettes.

Methyl red. [2]

[1] *Ber. dsch. chem. Ges.*, **35**, 2057 (1902).
[2] Prepared as in Kjeldahl determination, p. 89.

Course of the Analysis

After cleaning the apparatus with sulphuric-chromic acid and distilled water and drying thoroughly in the oven, the ground-in attachment is clamped in a cork, with the delivery tube horizontal, and the aluminium block is heated to 115°–120° C. For the chlorine determination, 4–6 mgm., and for the bromine determination, 5–8 mgm., of the substance to be analysed are placed on the bottom of the flask, using the weighing tubes with long handles. Liquids of low boiling point are best weighed in the very small weighing bottles (p. 106).

To the weighed material are added about 0·5 gm. of the dichromate mixture, from the tip of a knife. The joint for the stopper is moistened with rather concentrated sulphuric acid, the flask is closed, and the connection ensured by means of two steel springs. Two cubic centimetres of concentrated sulphuric acid are introduced into the funnel while the cock H_1 is closed ; the inlet tube is inserted and fixed. Now 1 c.c. of perhydrol is run into the absorption apparatus with a pipette, and about 7·5 c.c. of N/100 sodium hydroxide solution are added from the burette. The apparatus is assembled so that the inlet tube ends a few millimetres above the cock, H_2, but below the spiral.

The oxygen is drawn from a bomb by means of a needle reducing-valve. The bubble-frequency in a wash-bottle containing sodium carbonate solution is permanently adjusted by means of a Mariotte flask (p. 45), so that 8 c.c. of oxygen pass per minute.

The tubing of the wash-bottle is connected with the inlet tube of the funnel. After opening the cock H_1, the oxygen presses the sulphuric acid into the flask for the oxidation. The flask is then placed in the hole of the heating-block, where it remains for thirty minutes.

During this time the apparatus may be left and the weighing for the next determination may be made.

After the prescribed time, the cock H_1 is first closed and the tubing removed from the oxygen leading tube. A 100 c.c. silica flask is then placed under the outlet of the absorption apparatus, the alkali left to flow into this, and the apparatus washed with water afterwards. With the cock H_2 open, the mouth of the absorption apparatus is rinsed with about 4 c.c. of water, which is allowed to run out along the spiral. Without closing the cock, the apparatus is washed twice more with the same amounts of water, and finally the outlet is rinsed on the outside. For the titration, a small drop of methyl red is added with a glass thread and N/100 acid run in till a distinctly acid reaction is given. The carbon

dioxide liberated is then boiled off, another drop of methyl red added, and the solution titrated with N/100 sodium hydroxide solution to a canary yellow colour.

Calculation

From the total amount of alkali (N/100) used for the receiver and titration, there are subtracted the amount required for the perhydrol blank test and the amount of N/100 acid required for acidifying. One cubic centimetre of N/100 sodium hydroxide solution is equivalent to 0·3546 mgm. of chlorine or 0·7992 mgm. of bromine.

log (percentage of halogen) = log (atomic wt. of halogen) + log (c.c. of N/100 NaOH) — log (mgm. of substance weighed)

As previously stated, this method cannot be used for liquids of low boiling point and very volatile solids, because they are partly removed from the reaction. At the Kaiser-Wilhelm-Institut good halogen values were also obtained with substances containing nitrogen. Complete drying of the apparatus is most essential for successful analyses. With suitable storage no deterioration of the reagents is to be observed. The volumetric method represents a real advance on the gravimetric ; it permits the determination of the halogen in thirty-five minutes and is very easily carried out.

A new method for the determination of chlorides iodimetrically has been published by M. Berend.[1] The chloride is first precipitated in an alcoholic solution containing nitric acid, by means of silver nitrate solution. The surplus silver is then separated as iodate with excess of potassium iodate, and the iodate in the filtrate titrated with strong sodium thiosulphate after the addition of potassium iodide. According to the authors the process is accurate to ± 1 γ. The process fails if reducing compounds, e.g., sugar, are present.

Determination of Chlorine and Bromine in the presence of both, by the Method of L. Moser and R. Miksch [2]

The method depends on the thermal dissociation of the individual ammonium halides. If a mixture of silver chloride and silver bromide is volatilised with excess of ammonium iodide or bromide at 300° C., the total silver halide is transformed into silver iodide or bromide.

Course of the Determination

The organic substance is decomposed by the method of Pregl or Carius ; the silver halide precipitate is collected in a micro-Neubauer crucible as in the determination of sulphur (p. 117). This is dried

[1] *Biochem. Z.*, **252**, 362 (1932).
[2] " Pregl-Festschrift," p. 293, 1929.

at 150° C. and weighed. Dry ammonium iodide or bromide free from residue is then added to the crucible, using about six times the weight of the precipitate, and the crucible is heated, first covered and then open, in a muffle or in the aluminium block (p. 110) up to 250°–300° C., till all the ammonium salt has volatilised. After cooling on the copper block, the crucible is weighed. To check the constancy of weight, the heating with ammonium iodide is repeated.

From the weight of the silver chloride and bromide precipitate (first weighing) and the silver iodide or bromide formed (second weighing), the chlorine and bromine content of the substance is calculated from F. W. Küster's table 6.[1] The indirect determination of chlorine and bromine when both are present can be carried out accurately to ± 0·5 per cent.

Determination of Iodine by T. Leipert's Method [2]

Free iodine liberated in Pregl's spiral or bead-tube is collected in dilute sodium hydroxide solution and oxidised with bromine water to iodic acid. T. Leipert originally removed excess of bromine by passing in steam. According to F. Vieböck and C. Brecher,[3] formic acid has recently been used for the decomposition of the excess of bromine.[4] Potassium iodide is added to the iodic acid formed and the liberated iodine is titrated with N/50 sodium thiosulphate.

Reagents

Sodium Hydroxide Solution. Five grams of caustic soda (tablet form) are dissolved in 100 c.c. of water.[5]

Aqueous Sodium Acetate Solution. Made by dissolving 40 gm. of sodium acetate ($+ 3H_2O$), A.R., in 200 c.c. of water,[5] using a 5 c.c. pipette.

Solution of Sodium Acetate in Glacial Acetic Acid. Ten grams of sodium acetate ($+3H_2O$) are dissolved in 100 gm. of glacial acetic acid, using a 4 c.c. scale pipette.

Bromine, free from iodine, is kept in a dropping bottle under the hood.

Formic Acid (80-100 per cent.). Dropping bottle or dropping pipette.

[1] F. W. Küster : " Logarithmische Rechentafel für Chemiker, editions 35–40, p. 58, table 6, 1929.
[2] " Pregl Festschrift," p. 266, 1929.
[3] *Ber. dtsch. chem. Ges.,* **63**, 3207 (1930).
[4] Private communication from T. Leipert to A. Friedrich, p. 102. According to L. J. Goldberg, *Mikrochem.,* **14**, 161 (1934), the excess of bromine may also be removed by phenol.
[5] The distilled water must be carefully tested ; 50 c.c. should not use up any thiosulphate. The use of water which has been distilled twice is recommended for iodimetry.

Potassium Iodide (free from iodate). Ten per cent. aqueous solution [1] ; 2 c.c. pipette.

Sodium Thiosulphate, N/50. Fifty cubic centimetres of N/10 thiosulphate are run into a 250 c.c. graduated flask from a graduated pipette, 1·5 c.c. of amyl alcohol are added and the flask filled up to the mark. The factor for the solution is determined with N/20 potassium dichromate, as in the determination of isopropylidene groups (p. 209), using a micro-burette.

Sulphuric Acid, 2N ; 5 c.c. pipette.

Starch Solution, prepared as on p. 209.

Methyl Red, prepared as on p. 89.

Course of the Analysis

The spiral or bead-tube is cleaned and dried as on p. 89. For the iodine absorption, 4–5 c.c. of 5 per cent. caustic soda solution from a test-tube are aspirated through the neck of the tube to the upper edge of the spiral or bead layer. The solution is then allowed to run out, and the rinsed test-tube is placed over the end of the tube. The combustion is then carried out as on p. 101, using the platinum star contacts and 3–6 mgm. of the substance weighed in a boat or in a capillary. Oxygen passes through the wash-bottle at the rate of 3 c.c. per minute. The combustion should be finished in twenty-five to thirty minutes. Great care must be taken that, particularly during the decomposition of the substance, no iodine should separate behind the movable burner.   In such a case the coating of iodine, if it is not too near the rubber stopper, must be driven back again by slow heating.

Before the tube is left to cool in a stream of oxygen, it must be ascertained that all the iodine has been absorbed by the cautic soda. Iodine separated on the cooled wall of the tube between the tube burner and spiral is driven into the absorption solution by careful heating. Whilst the tube cools in a stream of oxygen, the weighing for the next determination is made and everything is prepared for the titration.

Five cubic centimetres of the 20 per cent. aqueous sodium acetate solution are pipetted into a 100 c.c. Erlenmeyer flask with ground-glass stopper. For the later rinsing of the tube, 4 c.c. of 10 per cent. sodium acetate solution in glacial acetic acid are taken and 2–3 drops of bromine added. After filling up the thiosulphate burette, the boat and the platinum contacts are taken out of the cooled tube, the roll of wire gauze removed, and the tube and test-tube are taken from the combustion stand. The prepared solution of brominated acetate in glacial acetic acid is poured into this test-tube

[1] See footnote, pp. 5, 113.

and aspirated into the combustion tube up to 1–2 cm. above the spiral or bead layer. Whilst closing the mouth of the tube with the finger, the neck is brought into the Erlenmeyer flask, the tube is then clamped at an angle of 60–70 degrees with the horizontal and the solution is allowed to flow out. It is then removed and about 6 c.c. of water from the test-tube are aspirated through and run into the Erlenmeyer flask. Finally the mouth of the combustion tube is rinsed twice with 6–8 c.c. of water from a wash-bottle, whilst rotating the tube.

Before the titration the bromine must be destroyed. Two to three drops of formic acid are run down the wall and the flask shaken carefully. If the solution is decolorised it is first tested for free bromine by the smell, then by adding a very small drop of methyl red with a glass thread. If the indicator is decolorised, bromine is still present and 1 drop more of formic acid must be added. If the solution remains slightly pink, 2 c.c. of the 10 per cent. potassium iodide solution and 5 c.c. of 2N sulphuric acid are added and allowed to stand five minutes with the flask stoppered. The iodine is then titrated rapidly, with N/50 thiosulphate, to a slightly yellow colour, 4–6 drops of starch solution are added, and the solution titrated to a slightly pink end-point, using methyl red as indicator ; 1 c.c. N/50 thiosulphate is equivalent to 0·4231 mgm. of iodine.

Calculation

log (percentage of iodine) = log (No. of c.c. N/50 $Na_2S_2O_3$ used) + log 0·4231 + 2 − log (mgm. of substance)

$$\log 0\cdot 4231 = \bar{1}\cdot 62644$$

Example :

5·492 mgm. iodobenzoic acid are equivalent to 6·55 c.c. N/50 $Na_2S_2O_3$

3·572 mgm.   ,,   ,,   ,,   ,, 4·32 c.c. ,,   ,,

Mol. wt. of $C_7H_5O_2I = 247\cdot 97$ $\begin{cases} \text{Theory, } 51\cdot 19 \text{ per cent. iodine.} \\ \text{Found, } 51\cdot 08 \text{ and } 51\cdot 17 \text{ per cent. iodine.} \end{cases}$

Because 1 atom of iodine in the substance yields 6 atoms for titration, with carefully prepared solutions very accurate results are obtained, which seldom deviate from the theoretical ones by more than ± 2 per cent. With analytically pure reagents and twice-distilled water no blank determination is necessary. The method has proved very good in various laboratories [1] and is decidedly preferable to the gravimetric.

T. Leipert [2] has briefly described a new process, which makes it possible to determine very small amounts of iodine in biological materials also (0·2–0·3 gm.) with greater accuracy. The organic

[1] A. Friedrich : "Die Praxis der quantitativen organischen Mikroanalyse," p. 103, W. Münster, *Mikrochem.*, **14**, 23 (1924).
[2] *Biochem. Z.*, **261**, 436 (1933).

substance is destroyed with sulphuric-chromic acid, using ceric sulphate as a catalyst ; the iodine is thus transformed into non-volatile iodic acid. Afterwards, the iodic acid is quantitatively reduced to iodine with arsenious acid and this is steam-distilled *in vacuo* into an alkaline solution, oxidised to iodate with bromine water, and titrated with standard thiosulphate (N/200–N/300), using starch as indicator.

IV. DETERMINATION OF SULPHUR
General

Sulphur in organic substances may be determined gravimetrically as well as volumetrically ; titration methods are always preferable to the combustion in the bead-tube.

The gravimetric determination may be carried out thus :—

1. By Pregl's method of combustion in the bead-tube, in which the products of combustion are absorbed in aqueous perhydrol, by which any sulphurous acid which may be formed is oxidised to sulphuric acid. The sulphuric acid is precipitated as barium sulphate and weighed.

2. By Pregl's modification of the Carius method.[1] The decomposition is carried out in the pressure tube with concentrated nitric acid and a crystal of barium chloride, barium sulphate precipitated, and weighed.

The basis of the volumetric methods is as follows :—

1. The sulphuric acid formed in the bead-tube may be titrated directly, by Pregl's method, with N/100 sodium hydroxide if nitrogen and halogens are absent.

2. A. Friedrich and O. Watzlaweck[2] have made it possible to titrate the sulphuric acid formed in the above combustion even in the presence of nitric, hydrochloric, and hydrobromic acids. To this end, all the acid is neutralised with sodium hydroxide in a quartz flask, with phenolphthalein as indicator. After the addition of exactly the equivalent amount of sulphuric acid the volatile acids are driven off by repeated evaporation on the water-bath, with formation of sodium sulphate, whilst the sulphuric acid originally present forms sodium bisulphate. Thus, if 1 gm. molecule of sulphuric acid and 2 gm. molecules of hydrochloric acid are present after the combustion, 4 molecules of sodium hydroxide are required for neutralisation.

$$H_2SO_4 + 2HCl + 4NaOH \rightarrow Na_2SO_4 + 2NaCl + 4H_2O.$$
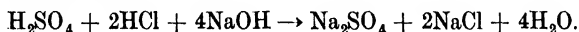
[1] Carius-Pregl : This process, like the halogen determination, is based on the investigations of F. Emich and J. Donaw : *Monatshefte f. Chem.*, **30**, 754 (1909).

[2] *Z. analyt. Chem.*, **89**, 401 (1932).

On evaporation with 2 gm. molecules of sulphuric acid, the following reaction occurs :—

$$Na_2SO_4 + 2NaCl + 2H_2SO_4 \rightarrow 2NaHSO_4 + Na_2SO_4 + 2HCl.$$
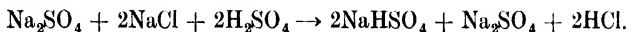
The titration of the bisulphate, therefore, corresponds with that of the original sulphuric acid.

Determination by Pregl's Bead-tube Method

The Micro-Neubauer Crucible

The barium sulphate precipitate is collected in a micro-crucible supplied by the firm of Heraeus, of Hanau, and weighed. The crucible is fitted with a lid and a capsule fitting over the bottom ; it is 14 mm. high and has a diameter of 12 mm. above and 10 mm. below. The filtering layer consists of compressed iridio-platinum sponge, which completely retains barium sulphate precipitated in the cold at a comparatively high rate of filtration.

We have occasionally come across crucibles, the filter-bed of which was unduly dense, so that the filtration was unnecessarily slow. Careful determinations show that a satisfactorily working micro-Neubauer crucible should pass an average of 4 c.c. of filtrate per minute by suction with the mouth. Before every determination, the inside of the crucible is cleaned under the tap with a small wad of cotton-wool wound round a match (not steel wire), and is then repeatedly washed with water after inserting it into the rubber collar of the filtering apparatus (p. 118). Very occasionally only, and after use for a long time, is it necessary to remove particles of barium sulphate held in the filtering layer by means of a little warm concentrated sulphuric acid. After cleaning the crucible in this manner it is advisable to filter on it a freshly prepared barium sulphate precipitate and to wash this thoroughly to ensure that the pores of the filter bed are properly closed. A Neubauer crucible with a filter-bed of porous porcelain, manufactured by the Berlin Porcelain Works, may also be used, but only one of the type in which the glaze extends beyond the rounded lower edge of the crucible, and not merely over the outer surface. If the lower edge and the adjacent parts are rough and unglazed, bad results are obtained, because on the one hand particles of the crucible are scraped off on pressing into the rubber collar, and on the other hand particles of rubber are retained in the rough porcelain wall.

The " Feather "

The transference of the barium sulphate precipitate from the dish into the crucible is carried out by means of the " Feather "[1]

[1] It is very resistant and stiff, and also of a shape which does not require modification for trimming.

(Fig. 39), in which a snipe feather is cemented into a thin glass tube. The glass tube, of 2–2·5 mm. external diameter and about 120–150 mm. long, is sealed at one end over the flame. As a cement for the feather, the glass cement is used which has already been described in the determination of carbon and hydrogen (p. 35) ; a small piece of this is stuck in the open end of the capillary and melted by warming on the regenerating block. The shaft of the feather is now pushed slowly into the glass tube, while keeping the latter warm. After mechanically removing the cement which adheres to the outside, the feather is washed quickly and consecutively in benzene, alcohol, and ammoniacal soap solution, by carefully rubbing it between the fingers. It is always kept under cover in a stoppered test-tube.

The Filtration Apparatus

For the collection of the barium sulphate precipitate, the apparatus used for the halogen determination is suitable. The crucible, T, is inserted in the rubber sleeve, M (Fig. 40). Because the filtration must be carried out with slight suction only, evacuation of the flask is carried out by suction with the mouth by means of a rubber tube, 50–60 cm. long, which is provided with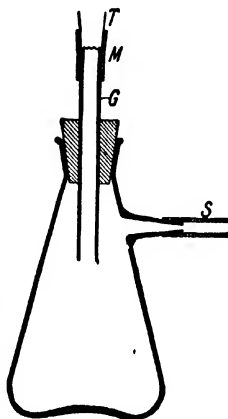 a pinchcock. With some experience, the pump may even be used. For this, a glass stop-cock is inserted in the pressure tubing, between the filter flask and pump, in the barrel of which fine grooves are scratched as in the connecting piece of the nitrogen determination (p. 78). A warning must be given about the regulation with pinchcocks, because it may easily lead to a sudden breaking of the vacuum.

Fig. 40.—Apparatus for filtration of barium sulphate. (⅓ actual size.)

T. Micro-Neubauer crucible. M. Rubber sleeve. G. Adjustable glass tube of 10 mm. outer diameter. S. Rubber tubing, 50 cm. long, with glass mouthpiece and pinchcock at end not shown in figure.

Fig. 39.— The "Feather." (⅓ actual size.)

O. Wintersteiner's [1] Automatic Filtration Apparatus

With this apparatus the barium sulphate precipitate is transferred to the platinum crucible by means of a syphon tube of narrow bore (0·8 mm.).

[1] *Mikrochem.*, **2**, 14 (1924).

Fig. 41 shows the apparatus in the form which has proved best. The micro-Neubauer crucible, *T*, is attached to a glass tube of 12·5 mm. outer diameter, inserted in a small filter flask in the usual manner by means of the rubber collar. The glass cover, *A*, is inserted over the crucible ; its wide, lower limb is put on over the slightly moistened rubber collar. The shorter limb of the capillary syphon tube is inserted into the upper end of this cover in such a manner that fairly large drops can form at its widened lower end, which should be placed at half the height of the crucible. A side-tube carries a rubber tube, *II*, about 50 cm. long, with glass mouth-piece and pinchcock, Q_2, while the side-tube of the filter-flask, *I*, is similarly provided, as usual. If the filter-bed of the crucible is
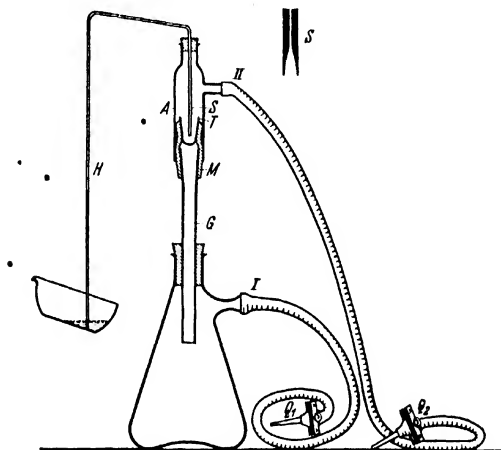

Fig. 41.—O. Wintersteiner's apparatus for automatic suction of barium sulphate.

initially dry and suction is applied at *I*, the vacuum extends through the filter-bed into the glass cover ; the liquid containing barium sulphate is aspirated through the syphon and on to the filter bed. Filtration and automatic transference of the precipitate proceed smoothly as long as the filter-bed of the crucible is covered by liquid. If the pressure should rise in the cover, *A*, so that the liquid which is transferred is no longer sufficient, the reduced pressure in the filter flask can no longer extend to the cover, as the now moist filter-bed offers too great a resistance. Careful suction with the mouth at *II*, after opening the pinchcock, Q_2, then restarts transference of the liquid and filtration recommences. Suction must not be too strong, or there is the danger that the liquid may creep over the edge of the crucible and that the determination may be spoilt by loss of the precipitate. When the contents of the dish have thus been trans-

ferred to the crucible, the dish is washed with water acidified with hydrochloric acid whilst filtration proceeds. The last traces of precipitate are transferred to the crucible in the usual manner by alternately rinsing the dish with alcohol and with water acidified with hydrochloric acid. The pinchcock, Q_2, is now opened, the glass cover removed, the syphon detached from the latter, and the shorter limb of the syphon well rinsed over the crucible.

Combustion and Precipitation of the Barium Sulphate

The combustion is carried out in the tube containing beads or spirals, as in Pregl's method for chlorine and bromine. Only the essential differences will therefore be discussed here.

The weight of material should be 4–7 mgm. The bead-tube is filled, by suction, with perhydrol which has been diluted with four times its volume of water. Ten cubic centimetres are used. The solution is then run into an Erlenmeyer flask and kept there for further determinations, which are made on the same day. The combustion tube is placed on the stand and the end covered with a clean test-tube. After charging the tube, a bubble velocity of 3 c.c. per minute of oxygen is set up and the combustion carried out as on p. 101. The movable burner is moved forward with more care, because the quantitative absorption of the sulphur trioxide in aqueous solution requires a rather long time of contact. The combustion requires thirty to forty minutes. A correspondingly larger amount of material must be used if the substance contains little sulphur. The time of combustion must then be increased to an hour or more.

After the tube has cooled in oxygen and the boat and the platinum stars have been removed, the tube is clamped at an oblique angle as in the halogen determination and rinsed into a Jena glass dish [1] of 7 cm. diameter or into a platinum dish.

The mouth of the combustion tube is rinsed three times with water acidified with hydrochloric acid (1 c.c. of conc. HCl per 100 c.c. of water), using as much acid each time as will cover the entire spiral or bead layer. The test-tube which was placed over the neck of the combustion tube is finally rinsed also.

To the united wash-waters in the dish, one or two crystals of barium chloride, weighing about 20 mgm., are added ; the solution is covered with a clock-glass with the convex side downwards and evaporated down on the water-bath. At first, decomposition of the perhydrol is observed and during this period the precipitation of the barium sulphate begins.

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[1] Dishes of ordinary glass must be steamed for several hours ; otherwise, with new dishes, too high sulphur values are obtained, owing to the separation of silica.

It is desirable to obtain as coarse a precipitate as possible for the filtration which follows. Evaporation is therefore taken completely to dryness and the residue taken up in a little water and then filtered.

Weighing the Crucible

Whilst the solution is concentrated on the water-bath, the crucible, cleaned as on p. 117, without the lower cover, is placed in the moistened collar of the filtration apparatus (Fig. 40), so that it rests on the glass tube, G. It is washed well with distilled water acidified with hydrochloric acid, removed from the collar with three fingers and closed with the lid and lower cap, and is then best placed on the lid of a large platinum crucible of about 30 mm. diameter, which lies on a silica triangle. It is first dried with a small flame, during which operation it often oscillates in consequence of the formation of steam. As soon as it is dry, it is heated to redness for three minutes with a stronger flame. The lid may also be held in the flame for a short period with platinum-tipped forceps in order to heat it to redness. The flame is turned out, and the crucible allowed to cool to not more than 150° C. It is then placed on a copper block which has been brought near it and transferred with this to the desiccator near the balance.

The time which must elapse before weighing may be shortened by transferring the crucible to a second copper block after cooling for a few minutes ; the crucible may then be weighed ten minutes after the ignition.[1] The crucible is transferred to the balance with a platinum-tipped forceps by which it is held in the centre without much pressure.

Filtration

The weighed crucible is returned to the copper block in the desiccator and carried in it to the filtration apparatus. For this purpose the lid and lower cap are removed, placed on the copper block, and the crucible placed in the moistened rubber collar of the filtration apparatus.

In the meantime, the barium sulphate precipitate has settled at the bottom of the dish. In order to transfer it to the crucible the dish is held in the left hand whilst the right holds the feather vertically over the middle of the crucible. The clear solution is then poured down the feather into the crucible, without disturbing the precipitate, until the crucible is full. It is aspirated through by the mouth or the pump (p. 118) and fresh solution added when almost all has

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[1] The crucible is always weighed with the lid and lower cap.

been run off. It is advantageous, during this somewhat delicate operation :—

1. To rub the outer edge of the dish, at the point of pouring, with the finger which has been slightly greased.

2. During decantation both elbows should be held close to the body, so that the edge of the dish and the feather are always maintained in contact above the centre of the mouth of the crucible.

3. The top of the liquid in the crucible should never be touched with the point of the feather, as in that case precipitate already in the crucible would creep back up the feather.

After the liquid has been decanted off, the dish is rinsed with a fine stream of water acidified with hydrochloric acid, using 1–2 c.c. ; and beginning at the edge, the precipitate is stirred up with the point of the feather and at once transferred to the empty crucible. The inner surface of the dish is again rinsed with water and is then rubbed with the feather from the edge to the centre and the contents once more poured into the crucible. The whole circumference of the inner surface of the dish is then rinsed with alcohol in a continuous stream, and the liquid once more transferred to the crucible down the feather. The crucible is then rinsed as before with water and the transference of the last, almost invisible, particles of precipitate assisted by rubbing with the feather. The rinsing of the dish with alcohol and water alternately is repeated at least once more. With a little practice the last residues will be removed by the double utilisation of the surface tension between alcohol and water. The beginner will find that a rather longer time is needed. It is always necessary to use water for the final washing for, if the precipitate and the filter layer are left moistened with alcohol, violent spirting of the precipitate may occur on subsequent heating, which may even damage the filtering layer.

The suction of the barium sulphate precipitate by O. Wintersteiner's method is described on p. 118. At the end of either method of suction—the choice of which is left to the individual—the crucible is washed twice more with acidified water, the lid and lower cap replaced, ignited as on p. 121, and cooled.

To remove any barium chloride carried down with the barium sulphate precipitate, the crucible is replaced, after ignition, in the moistened rubber collar, washed three times with acidified water, ignited as before with the lid and lower cap, and weighed accurately to 0·005 mgm. as before. It is advisable to control the weight by once more washing, igniting, and weighing. As a rule this results in a loss in weight of not more than 0·005 mgm., or at most 0·01 mgm., if large quantities of water are used.

1 mgm. of sulphur is equivalent to 7·281 mgm. $BaSO_4$.

Calculation

log (percentage of S) = log (wt. BaSO$_4$) + log (factor)
+ 2 − log (wt. of substance).

$$\text{Factor } \frac{S}{BaSO_4} = 0\cdot1373. \quad \text{Log (factor)} = \bar{1}\cdot13282.$$

Examples :

	Sulphonal.	Barium sulphate.	Percentage found.
(a)	3·876 mgm.	7·49 mgm.	28·14
(b)	6·1875 mgm.	12·745 mgm.	28·31

Molecular wt. of sulphonal, C$_7$H$_{16}$O$_4$S$_2$ = 228·24.

∴ Theoretical percentage = 28·09 per cent.

Determination of Sulphur by Carius' Method

According to F. Pregl and M. de Crinis,[1] if organic material is decomposed with concentrated nitric acid without the addition of barium chloride, slight losses in sulphur easily occur on opening the tube. If the pressure tube is heated up to 280°–300° C. for five hours with conc. nitric acid in the presence of powdered barium chloride [2] (10 to 20 mgm.), losses still occur frequently, even when using glass of good quality. The loss of barium sulphate was ascribed to the fusion of barium sulphate into the glass, so that it could not be removed even by carefully rubbing with a feather.

Also, F. Pregl [3] has drawn attention to the too high values which occur through the separation of silica from the glass. He detected silica even when a solution of barium chloride was concentrated in a glass dish. With the decomposition in the pressure tube, the conditions for the separation of silica are naturally more favourable, even if good glass is used.

Therefore, while it is permissible as a rule, though not always, to determine chlorine and bromine by the micro-Carius method, errors easily occur in the determination of sulphur if the following precautions are not observed [4] :—

1. New Jena glass (red streak) pressure tubes must always be used.

2. The temperature should not rise above 270° C.

3. The addition of too much barium chloride (*i.e.*, of more than 15 mgm.), must be avoided.

From the pressure tube, opened by J. Unterzaucher's method

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[1] Second (English) edition of this book, p. 150.
[2] Powdered in order to avoid occlusion of BaCl$_2$.
[3] Second (English) edition of this book, p. 139.
[4] According to private communications from J. Unterzaucher.

(p. 107), the precipitate is rinsed out quantitatively with acidified water into a clean crystallising dish and then treated as on p. 120.

Volumetric Determination of Sulphur

Pregl's Method for Substances Free from Nitrogen and Halogens

The substances are burnt in the bead-tubes, as in the gravimetric method (p. 120), and the products of combustion are absorbed in a perhydrol solution (perhydrol to water, 1 : 5), which has been neutralised with N/100 sodium hydroxide to a canary-yellow colour, using methyl red as indicator.

After the combustion, the bead-tube is rinsed, as on p. 147, with distilled water, the washing-water is collected in a 100 c.c. wide-necked quartz flask, a small drop of methyl red is added with a glass thread, and the sulphuric acid formed is titrated with N/100 alkali to the canary-yellow colour. During this titration account must be taken of the fact that the solution subsequently turns pink. The burette reading is therefore only taken when the canary-yellow colour has been maintained for two minutes.

One cubic centimetre of N/100 sodium hydroxide solution corresponds to 0·1603 mgm. of sulphur; log factor = $\bar{1}$·20493. By this method it is possible to determine sulphur quickly and accurately, and it is preferable to the gravimetric method if nitrogen and halogens are absent.

Method of A. Friedrich and O. Watzlaweck [1] for Substances containing Nitrogen, Chlorine and Bromine

The combustion is carried out in the bead-tube, as described in F. Pregl's volumetric determination of sulphur; the absorption solution and the washings are collected in a platinum or quartz dish. Only clear and transparent rock crystal dishes may be used. After adding 1 drop of phenolphthalein to the solution, N/50 sodium hydroxide solution is run in from a burette till a clear red colour is obtained, whilst stirring with a glass rod, and the reading taken. The dish is then placed half inside the water-bath. After reading, the burette is filled up and the bead-tube cleaned for the next determination.

After twenty to thirty minutes, the concentrated sodium sulphate solution is removed from the water-bath, and N/50 sulphuric acid is run in from a burette, using an amount equivalent to the N/50 alkali previously taken. If the contents of the dish have been evaporated to dryness, this has no harmful influence on the results.

The solution is stirred well with a rinsed glass rod after the addition of sulphuric acid and evaporated to dryness on the water-bath. The residue is taken up in a little water run in from a wash-

[1] *Z. analyt. Chem.*, **89**, 401 (1932).

bottle in a thin stream along the edge of the dish, and again evaporated completely to dryness. Water is then again run on to the crust of salt, and the residue allowed to stand for forty-five minutes on the boiling water-bath. Evaporation three times is essential to drive off the volatile acids and to convert the sulphuric acid originally present into the sodium bisulphate to be titrated.

• For the titration, the residue is taken up with 5–8 c.c. of hot water, methyl red added, and the solution titrated with N/50 sodium hydroxide solution to the first appearance of the yellow colour ; the dish is then placed for some minutes on the water-bath and titrated still hot to the canary-yellow colour.

Because in practice it is never certain if all volatile acids have been completely removed before the bisulphate titration, a control titration must be made. If this is found to be superfluous, it may be omitted in further analyses of the same or similar substances.

For the control test, an amount of N/50 sulphuric acid equivalent to the total alkali used for neutralisation and for the bisulphate titration ·is run into the dish containing sodium sulphate. The dish is evaporated to dryness on the water-bath, the residue taken up with water twice as before, allowed to remain on the water-bath for forty-five minutes, and titrated as above with N/50 alkali. If all the volatile acids have been removed in the first determination, all the sulphuric acid added in the control test will be titrated back, otherwise, a proportionately small amount of acid will be required.

$$1 \text{ c.c. N/50 NaOH} = 0.3206 \text{ mgm. sulphur.}$$

$$\text{Factor} = 0.3206. \quad \text{Log (factor)} = \bar{1}.50596.$$

$$\text{Log (percentage of sulphur)} = \log (\text{c.c. N/50-NaOH}) + \log F$$
$$+ 2 - \log (\text{milligram of substance}).$$

Substances containing iodine cannot be titrated by this method, because too low values would be obtained. Three to four hours are necessary for an analysis, which is accurate to $\pm$ 0.2 per cent.

Of the different methods used for the determination of sulphur, Pregl's gravimetric bead-tube method has always yielded excellent results at the Kaiser-Wilhelm-Institut. Originally we used simple platinum contacts, prepared by ourselves, with which too low results were occasionally obtained, owing to incomplete oxidation. When the platinum contacts made by the firm of C. Heraeus (Fig. 30) were used, this error no longer occurred. This method is particularly to be preferred in the case of substances of unknown constitution.

The sulphur in substances which only contain carbon, hydrogen and oxygen besides, may be determined in less time with equal accuracy by F. Pregl's volumetric bead-tube method.

The bisulphate titration naturally takes up time but is not inferior to the previous methods in accuracy and is to be strongly recommended for microanalytical pupils. In the scientific work of the micro-laboratory, where we have to reckon with the presence of the most various elements, the general gravimetric process is given preference.

Determination of Halogens and Sulphur Simultaneously by A. Friedrich's Method [1]

The organic substance is decomposed in the pressure tube with nitric acid and a crystal of silver nitrate as in Carius' method. The filtrate from the silver halide precipitate and the washings are collected in a short test-tube placed in the filter flask, rinsed quantitatively into a Jena glass dish, and the sulphuric acid precipitated on the water-bath with 2–3 c.c. of a 1 per cent. solution of barium nitrate. This solution must be tested for „halogen with silver nitrate. If it should not be free from halogen, this must be precipitated from the warm solution with a little silver nitrate and the filtrate added to the solution in the dish.

The barium sulphate precipitate is filtered and washed with distilled water which has not been acidified. The filtration and weighing are carried out as on p. 121. The method gives satisfactory results.

V. DETERMINATION OF PHOSPHORUS BY H. LIEB'S METHOD [2]

Principle

There are many methods by which small amounts of phosphorus may be very accurately determined as phosphoric acid gravimetrically, volumetrically, colorimetrically, and nephelometrically. Those which have proved best for the analysis of crystallised organic compounds may here be described.

The organic substance is destroyed either by fusion with sodium carbonate and saltpetre or by boiling with a mixture of nitric and sulphuric acids, by which the phosphorus is oxidised to phosphoric acid. The acid is quantitatively precipitated with the ammonium-molybdate reagent of N. v. Lorenz [3] and weighed as ammonium phosphomolybdate or determined volumetrically by dissolving the precipitate in excess of standard sodium hydroxide solution, boiling off the ammonia, and titrating back the excess of alkali.

[1] " Die Praxis der quantitativen organischen Mikroanalyse," p. 109.
[2] Second (English) edition of this book, p. 151; also H. Lieb and O. Wintersteiner, *Mikrochem.*, **2**, 78 (1924).
[3] *Z. analyt. Chem.*, **51**, 161 (1912).

An analysis accurate to $\pm$ 0·1 per cent. is easily attained, because the precipitate weighed is sixty-three times as heavy as the phosphorus which it contains or thirty times as heavy as the phosphorus pentoxide.

The conditions of preparation of the reagents and of precipitation must be observed most strictly, because the factor of N. v. Lorenz,[1] H. Lieb[2] and R. Kuhn[3] has been empirically ascertained and holds only for the method worked out for the given conditions.

With the titration carried out according to P. Iversen's equation,[4] 1 atom of phosphorus requires 28 equivalents of alkali.

Reagents

Sulphate—Molybdate Reagent. Fifty grams of ammonium sulphate are dissolved in 500 c.c. of nitric acid, of specific gravity 1·36, in a graduated litre flask ; 150 gm. of powdered ammonium molybdate are dissolved in 400 c.c. of boiling water in a beaker. After cooling, the latter solution is poured slowly, with stirring, in a thin stream into the former. The mixture is then filled up to the mark, allowed to stand for three days, and filtered through an ordinary filter into a brown·bottle which is kept well stoppered.

Dilute Nitric Acid (1 : 1).

Nitric Acid containing Sulphuric Acid. Thirty cubic centimetres of sulphuric acid (specific gravity 1·84) are poured into 1 litre of nitric acid (specific gravity 1·19–1·21), which is obtained by mixing 420 c.c. of nitric acid (specific gravity 1·40) with 580 c.c. of water.

A 2 per cent. Aqueous Solution of Pure Ammonium Nitrate. Should the solution not be weakly acid it must be slightly acidified with a few drops of nitric acid.

Pure Alcohol, 95–96 per cent. by Volume.

Ether. This should be free from alcohol and water. At room temperature, 150 c.c. of ether should dissolve 1 c.c. of water to form a clear solution.

Acetone (A.R.). This must be free from aldehydes.

Soda-saltpetre Mixture. Purest, finely-powdered sodium carbonate and potassium nitrate mixed in the proportion 1 : 1.

For destruction in the wet way, sulphuric acid (specific gravity 1·84), nitric acid (specific gravity 1·4), and E. Merck's acid-free perhydrol are required.

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[1] See footnote 3, previous page.
[2] See footnote 2, previous page.
[3] *Z. physiol. Chem.*, **129**, 64 (1923).
[4] *Biochem. Z.*, **104**, 23 (1920).

Gravimetric Determination

Decomposition with Fused Sodium Carbonate and Sodium Nitrate

The substance is weighed into a platinum boat, using 2–5 mgm., and covered with five to six times its weight of the mixture of sodium carbonate and sodium nitrate; these are well mixed by means of a thin platinum wire, which is then left in the boat. The boat is placed in a combustion tube of Supremax glass, about 15 cm. long, which has been carefully cleaned with sulphuric-chromic acid. The tube is bent at right angles at the end which is drawn out to a capillary. The other end is closed by a rubber stopper; oxygen is passed through a narrow capillary in this at the rate of 3–4 c.c. per minute. After the oxygen is introduced, the tube is first heated in front of the boat and the flame gradually advanced up to the boat. Immediately the main reaction is over, the boat is heated with the full, non-luminous flame underneath and then allowed to cool in the current of oxygen.

The boat is then brought into a test-tube, the fused mass dissolved by boiling in about 5 c.c. of dilute nitric acid 1 : 1 and filtered through a moistened hard filter into a 100 c.c. test-tube cleaned with sulphuric-chromic acid. If particles of the melt have spirted into the combustion tube, this must also be rinsed out with hot dilute nitric acid (1 : 1) introduced through its mouth whilst holding obliquely.[1] When the material is dissolved, the clear solution is run out through the capillary whilst rotating the tube.

To the cooled filtrate, 2 c.c. of nitric acid containing sulphuric acid are added, and also water if necessary, to dilute the total volume to 15 c.c.

Decomposition with Acid

The substance is weighed into a small dry Kjeldahl flask by means of a nitrogen weighing tube, using 3–5 mgm. of material; the flask has been cleaned with sulphuric-chromic acid. To this acid, 3–5 mgm. of material are taken. To this are added 0·5 c.c. of concentrated sulphuric acid (specific gravity 1·84) and 4–5 drops of conc. nitric acid (specific gravity 1 : 4); it is then heated over a small flame until SO_3 fumes are evolved and this process repeated twice more from the addition of the nitric acid. If, after cooling, the solution is still not clear, 4–5 drops of perhydrol are added and the material again heated until SO_3 fumes appear. This is repeated until the solution is quite clear, when the flask is rinsed into a wide-necked test-tube and treated further as above.

The test-tubes of the solutions obtained in both cases are placed

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[1] R. Kuhn was able to avoid spirting entirely with preparations of saccharase, peroxidase, and lipase, when the carbon was first heated in the current of air and then burnt in oxygen.

in a litre beaker on the boiling water-bath. Meanwhile, the molybdate reagent is filtered. For the precipitation, the test-tube is removed from the water-bath and 15 c.c. of the reagent is run into it from a pipette without touching the side. After two to three minutes the precipitate is shaken well, then left to stand for at least six hours to precipitate the ammonium phosphomolybdate completely. According to R. Kuhn,[1] for the quantitative separation of the precipitate with less than 0·5 mgm. of phosphorus six to eighteen hours, and for less than 0·05 mgm., up to thirty-six hours, are required.

It should be noted that after precipitation is completed the test-tube must not be heated again on the water-bath, else precipitation of free molybdic acid would occur.

During the separation of the precipitate, a new filter-tube (p. 98) is prepared in the filter-flask, or else one which has already been used is cleaned by dissolving the ammonium phosphomolybdate with ammonia. It is washed, in either case, with water, hot dilute nitric acid, and distilled water, and the water is finally removed by filling up twice with alcohol, ether, or acetone. The filter-tube is then wiped with moist flannel and dry chamois leather and left for at least half an hour in an empty desiccator, containing no drying agent, which is evacuated with the water pump. The disappearance of all smell of ether or acetone indicates that the tube is ready for weighing.

The filter-tube is removed from the desiccator immediately before use and the time noted. Five minutes should elapse between this and the weighing of the tube. Later, the weighing of the precipitate should be carried out after exactly the same interval.

The precipitate is drawn into the filter-tube by means of the same automatic arrangement as is used for the halide precipitate (p. 98). After the supernatant liquid has been syphoned off, the precipitate is washed thoroughly with the 2 per cent. ammonium nitrate solution and transferred to the filter. To remove the last traces of precipitate the walls of the test-tube are rinsed round well with ammonium nitrate solution and 95 per cent. alcohol alternately and the tube is finally filled twice with alcohol and ether or acetone to remove the water. After the wiping with flannel and chamois leather, the tube is weighed under the same conditions as before. Because the weight of the ammonium phosphomolybdate is much greater than that of the phosphorus contained in it, weighing to ± 0·01 mgm. is sufficiently accurate.

Calculations

The weight of the precipitate multiplied by the empirically

[1] *Z. physiol. Chem.*, **129**, 64 (1923).

determined factor, $F = 0.01454$, gives the weight of phosphorus. Log $F = \bar{2}\ 16209$.

$$\log (\text{percentage of P}) = \log (\text{weight of precipitate}) + \log F$$
$$+ 2 - \log (\text{weight of substance}).$$

Example :   *Diphthaloyl-phosphoric Acid Ester.*   5·925 mgm. yielded 36·08 mgm. of ammonium phosphomolybdate.

Theory for $C_{20}H_{13}PO_4$ (mol. wt. 348·1) : P = 8·91 per cent.
Found : P = 8·81 per cent.

Volumetric Determination

This method has been developed from Blair's alkalimetric determination of phosphoric acid in iron and steel.   At first, the method is identical with the gravimetric determination, only the precipitation is made in a 100 c.c. Jena beaker.   After standing overnight, the supernatant liquid is poured through a hard filter, and the precipitate washed with a little ice-cold 50 per cent. alcohol.   The particles on the filter are carefully rinsed back into the beaker.

The precipitate is dissolved in N/10 alkali, using twice as much as will just dissolve it, and ammonia is boiled off for at least half an hour.   The final volume is about 10 c.c.   To the alkaline solution, 2–3 drops of 1 per cent. phenolphthalein solution are added (or thymophthalein may be used), about 3–5 c.c. excess of N/10 acid are run in, the solution is boiled for ten to fifteen seconds, cooled, and titrated back with N/10 sodium hydroxide.

The phosphoric acid present is calculated according to P. Iversen's [1] equation :

$$(NH_4)_3PO_4 . 12MoO_3 . 2HNO_3 + 28NaOH =$$
$$12Na_2MoO_4 + 2NaNO_3 + Na_2HPO_4 + 16H_2O + 3NH_3.$$
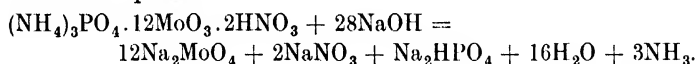
The N/10 alkali used, multiplied by the factor $F_1 = 0.1107$, gives the amount of phosphorus in milligrams.   log $F = \bar{1}.04415$.

As a rule the gravimetric method [2] is to be preferred to the volumetric, although the latter also yields very accurate results, because the rinsing and evaporation is slower than the filtration and weighing.

The method of G. Embden [3] differs from the others described in that the phosphoric acid is precipitated as strychnine phosphomolybdate.   The ratio of phosphorus to strychnine phosphomolybdate is 1 : 98.   Because the precipitation can be carried out in the cold, is practically quantitative in a short time, and can be filtered after only half an hour, it is very suitable for physiological

[1] *Biochem. Z.*, **104**, 23 (1920).
[2] For the determination of small amounts of phosphorus (less than 0·1 mgm.), R. Kuhn absolutely prefers the gravimetric method.
[3] *Z. physiol. Chem.*, **113**, 138 (1921).

determinations especially ; the danger of liberation of free phosphoric acid from organic phosphates in many cases is reduced to a minimum.

If the organic substance also contains arsenic, then a mixture of phosphoric and arsenic acids is obtained by the decomposition. The latter must be removed before precipitation. R. Kuhn [1] has worked out a micro-separation of phosphoric acid and arsenic acid. The pentavalent arsenic is reduced with hydrazine in hydrochloric acid and the trichloride distilled off in a slow stream of hydrogen chloride. According to whether the phosphorus is determined gravimetrically in nitric acid or nephelometrically in hydrochloric acid, it is evaporated down with nitric or hydrochloric acid.

For other micro-methods for the determination of phosphorus, reference should be made to the reports of F. Feigl, R. Strebinger and H. K. Barrenscheen.[2]

Simultaneous Determination of Barium and Phosphorus [3]

In barium salts of organic esters of phosphoric acid, barium cannot be determined by evaporation to fuming with sulphuric acid. According to the following method of analysis the barium and phosphorus may be determined on one weight of substance with the required degree of accuracy (0·2 per cent.). About 2-5 mgm. of the substance are weighed from the long-handled weighing tube (p. 79) into a carefully cleaned and dried small Kjeldahl flask ; 0·5 c.c. of concentrated sulphuric acid (specific gravity 1·84) is added with a scale pipette. Using a wooden holder, the substance is decomposed over the smallest possible Bunsen flame. For multiple determinations the combustion stand (p. 89) may suitably be used. After the flask has been heated slowly till fumes of sulphur trioxide appear, it is allowed to cool and from 5 to 8 drops of perhydrol (p. 100) are added from a dropping pipette and the flask reheated till the perhydrol is destroyed and sulphur trioxide fumes escape at the neck of the flask. This process is repeated until the solution is quite clear, which is usually after three additions of perhydrol. The barium sulphate is in solution in the anhydrous sulphuric acid.

The flask is then allowed to cool and is rinsed carefully, whilst shaking, with a fine stream of 3 c.c. of distilled water along the wall into the sulphuric acid, when the barium sulphate is precipitated immediately. The contents of the flask are then rinsed quantitatively, with a further 3 c.c. of water, into a Jena glass crystal-

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[1] *Z. physiol. Chem.*, **129**, 64 (1923).
[2] *Mikrochem.*, **7**, 116 (1929).
[3] Unpublished.

lising dish which has been cleaned with sulphuric-chromic acid, and *this is covered with a clock-glass.*

Meanwhile, the Neubauer platinum crucible is cleaned, ignited, and weighed for the filtration of the barium sulphate (p. 117). For preference, a wide-necked filter flask is here used, in which a glass insertion for the collection of the filtrate is placed. This consists of a wide preparation glass of 20–25 c.c. content. After the filter flask has been cleaned and moistened with distilled water, the crucible is inserted in the rubber collar and the precipitate brought into it as on p. 121. The barium sulphate can be rinsed into the crucible without difficulty with 2 c.c. of distilled water. When the crucible has been emptied by suction, the vacuum is turned off, the crucible rinsed with about 1 c.c. of water, in order to remove the last traces of solution, and suction applied again for two minutes.

The crucible is now raised somewhat out of the collar and the bottom of the crucible and the collar are rinsed inside with 1–2 c.c. of water. The crucible, with its lower cap and lid, is placed on a large platinum lid and ignited and weighed as on p. 121.

The glass insertion with the solution and washings, 10–11 c.c. altogether, is taken out of the flask with a forceps, the solution rinsed into a wide test-tube with 2–3 c.c. of water, and the ammonium phosphomolybdate is precipitated in it by the addition of 2 c.c. of nitric acid containing sulphur, as on p. 128.

VI. DETERMINATION OF ARSENIC

Lieb's Gravimetric Method [1]

The organic substance is either decomposed in a small pressure tube with nitric acid or destroyed in a small Kjeldahl flask with sulphuric acid and perhydrol. The arsenic acid formed is precipitated with magnesia mixture as magnesium ammonium arsenate and weighed after ignition as magnesium pyroarsenate.

Course of the Analysis

(a) *Decomposition in the Pressure Tube.* With the weighing tube with the long handle (p. 79), 5–10 mgm. of the substance are weighed into a small pressure tube cleaned with sulphuric-chromic acid ; about 0·5–1 c.c. of conc. nitric acid is added from a pipette whilst rotating the tube. The tube is sealed in the blowpipe as on p. 106, and heated for several hours in the small tube-furnace to

[1] H. Lieb : From Abderhalden's *Handbuch der biochemischen Arbeitsmethoden,* **1, 3,** 388 (1921).   Also H. Lieb and O. Wintersteiner : *Mikrochem.,* **2,** 80 (1924).

250°–300° C., according to the ease with which the substance is decomposed. After opening (p. 107), the contents of the tube are rinsed quantitatively with distilled water into a glass dish of 30–40 c.c. capacity and the separated portion of the tube is also rinsed carefully.

(*b*) *Decomposition in the small Kjeldahl Flask.* The substance is transferred from the long-handled weighing tube to the bottom of the dry flask and treated with 5 drops of 30 per cent. sulphuric acid and 0·5 c.c. of perhydrol. It is heated as on p. 128, if necessary with fresh addition of perhydrol. When all the material is decomposed and the solution is clear, the solution is rinsed quantitatively into the glass dish.

Precipitation and Weighing. The solution is evaporated to dryness on a water-bath and the residue dissolved in 3–4 c.c. of 2 N-ammonia, and 1 c.c. of magnesia mixture added from a pipette. This is prepared by dissolving 5·5 gm. of crystallised magnesium chloride and 10·5 gm. of ammonium chloride in 100 c.c. of water. As the precipitate which separates is amorphous at first and filtration is very difficult, it must be allowed to stand for at least six hours, after which it is crystalline.

The magnesium ammonium arsenate is filtered into a micro-Neubauer crucible (p. 117) with the help of the feather, as described in the determination of sulphur (p. 121), and washed with 2 N-ammonia. The last traces are transferred to the crucible by the alternate use of 2 N-ammonia and alcohol. The filtration may also be carried out by O. Wintersteiner's automatic method (p. 119). The crucible is then removed from the rubber collar, closed with the lid and lower cap, and strongly ignited on a large platinum lid. The magnesium pyroarsenate thus obtained still occludes magnesium salts and must therefore be washed thoroughly with very weakly ammoniacal water. After again igniting strongly the crucible is brought to the balance on the copper block of the micro-desiccator and may be weighed after ten minutes. Further washing usually results in a loss of weight of 0·01 mgm. only. The method leads to very accurate results, which differ from the theoretical ones by 0·2 per cent. at most, and are usually slightly too low.

Calculation

$$\log \text{(percentage of arsenic)} = \log \text{(wt. of } Mg_2As_2O_7)$$
$$+ \log 0·4827 + 2 - \log \text{(wt. of material)}$$
$$0·4827 = \text{Factor} = \frac{As}{Mg_2As_2O_7}$$
$$\log 0·4827 = \bar{1}·68368.$$

O. Wintersteiner's [1] Volumetric Method

The volumetric method of determination of arsenic represents a real advance. It is especially advisable for multiple determinations. Oxidation of the organic substance occurs in a very short time in the wet way in a small Kjeldahl flask with sulphuric and nitric acids and perhydrol. The arsenic acid formed is treated in hydrochloric acid with potassium iodide and the liberated iodine titrated with N/100 thiosulphate solution. The reaction is :

$$2HI + H_3AsO_4 = H_2O + I_2 + H_3AsO_3.$$
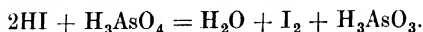
Reagents

Sulphuric Acid. Thirty per cent.

Nitric Acid. Specific gravity 1·4.

Perhydrol. E. Merck's acid-free.

Hydrochloric Acid (conc.). About 25 c.c. are boiled gently for exactly two minutes in a 100 c.c. Erlenmeyer flask, to remove free chlorine. The ground-in stopper is then immediately inserted and the flask cooled under the tap. The cold acid is put in a burette.

Potassium Iodide Solution. Four per cent., 150 c.c. The solution must be colourless.

Thiosulphate Solution. N/100. Twenty-five c.c. of ripened N/10 thiosulphate solution are placed in a 250 c.c. graduated flask, 2·5 c.c. of amyl alcohol, A.R., added, and the flask filled up to the mark with boiled-out water. The solution is stored in a brown bottle and the factor determined after one or two days with N/50 chromic acid [2] or potassium hydrogen iodate, as on p. 209. The potassium hydrogen iodate is weighed into an Erlenmeyer flask, using 2·5-3 mgm., dissolved in 5 c.c. of water which has been boiled, and 3 c.c. of the above conc. hydrochloric acid and 2 c.c. of the 4 per cent. potassium iodide solution are added. After two minutes the liberated iodine is titrated. Towards the end of the titration the liquid is diluted to 20 c.c., starch solution added, and the titration completed.

Starch Solution. Prepared as on p. 209.

Course of the Analysis

For the analysis, 7-12 mgm. of the substance are weighed into a small dry Kjeldahl flask as on p. 90 ; particles which adhere to the wall are rinsed down into the bulb with 1 c.c. of 30 per cent. sulphuric acid. After adding 4 or 5 drops of concentrated nitric

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[1] *Mikrochem.*, **4**, 155 (1926).

[2] For acidifying, 3 c.c. of concentrated hydrochloric acid are used instead of sulphuric acid.

acid the flask, in a wooden holder, is heated over a small flame. For multiple determinations, the samples can be heated on the combustion stand. Immediately white sulphur trioxide fumes appear, this process is repeated after adding the same amount of nitric acid. Finally, 5 drops more of perhydrol are added. The solution usually remains clear on cooling. If the substance is very difficult to burn, the addition must be repeated until the solution is quite clear. After cooling, in order to remove the hydrogen peroxide completely, and more particularly to destroy excess of oxyper-sulphuric acid, the liquid is evaporated two or three times with 1 c.c. of water each time, until SO_3 fumes appear and strong sulphuric acid condenses on the wall of the flask. After again adding 1 c.c. of water the flask is boiled for a moment and its contents poured into a wide-necked bottle, or, better, a wide-necked 100 c.c. Erlenmeyer flask. For quantitative rinsing of this flask, 5 c.c. of pure, boiled-out hydrochloric acid are used.

For the titration, 2 c.c. of potassium iodide solution are added and the mixture allowed to stand for ten minutes in the flask, closed by a ground-in stopper. The liberated iodine is titrated with $N/100$ thiosulphate solution from a 10 c.c. micro-burette with glass cock. When the solution is only faintly yellow, the liquid is diluted to the 20 c.c. mark with boiled-out water, 4–5 drops of starch solution are added and the titration completed. A faintly reddish tint is to be considered the end point ; the blue colour only reappears after five to ten minutes.

The values for arsenic obtained by this method for substances containing bromine, and still more for those containing iodine, are too high. To remove iodic acid, 0·3 c.c. of the above solution of potassium iodide and 1 c.c. of water are placed in the flask after the oxidation and destruction of the oxypersulphuric acid are complete. The liquid is heated till all the iodine is volatilised, after which it is heated again with perhydrol till SO_3 fumes appear, in order to oxidise the partially reduced arsenic acid. Finally, it is evaporated twice, each time with 1 c.c. of water, to destroy the perhydrol and oxypersulphuric acid.

Blank Test

In spite of the use of boiled-out solutions only, a small excess of iodine is usually obtained owing to the oxidising action of the air on the reagents. It is therefore necessary to carry out a blank test on the reagents before every series of determinations. To 1 c.c. of 30 per cent. sulphuric acid in a test-tube, 1 c.c. of water is added, the mixture boiled and the contents rinsed into the titration vessel

with 5 c.c. of boiled hydrochloric acid. Two c.c. of the 4 per cent. solution of potassium iodide are added and the liquid allowed to stand for ten minutes in the stoppered flask, after which it is diluted to the 20 c.c. mark, starch is added, and the solution titrated to the faint reddish colour with N/100 thiosulphate.

The quantity of thiosulphate solution required in the blank test varies from 0·04 to 0·08 c.c., and must be deducted from that found in the actual determination.

Calculations

As 1 atom of arsenic corresponds to 2 atoms of iodine, 1 c.c. of N/100 thiosulphate corresponds to 0·3748 mgm. of arsenic.

$$\text{Log } 0\cdot3748 = \bar{1}\cdot57380.$$

log (percentage of As) = log (c.c. N/100 thiosulphate)
$$+ \log 0\cdot3748 + 2 - \log \text{(milligrams of substance)}.$$

Example : 9·417 mgm. of phenyl-arsenious acid require 9·36 c.c. of N/100 $Na_2S_2O_3$.

∴ Percentage of Arsenic : $\begin{cases} \text{Theory, } 37\cdot18. \\ \text{Found, } 37\cdot24. \end{cases}$

For further methods for the determination of arsenic, see references of K. Heller, *Mikrochem.*, **14**, 369 (1934).

VII. DETERMINATION OF METALS IN ORGANIC COMPOUNDS BY F. PREGL'S METHODS

This determination is one of the simplest micro-analytical methods, and does not differ in principle from the macro-processes. Organic salts of metals, if the metals are not to be determined simultaneously with the carbon and hydrogen as on p. 65, are ignited in a platinum or porcelain crucible or in a small boat. Thus, according to the following processes, are determined :

(*a*) Metallic oxides as residues, weighed after direct ignition.

(*b*) Metals weighed as sulphates, after evaporating down with sulphuric acid.

The crucible with lid is about 15 mm. high, with an upper diameter of 12 mm. and a lower one of 10 mm. The residue remaining from previous determinations is removed by cleaning with dilute or conc. nitric acid, sulphuric acid, fused potassium sulphate, and water. The platinum crucible is ignited on a rather large platinum lid, of 30–40 mm. diameter, which protects it from direct contact with the gases of the flame ; this lid rests on a silica triangle. For the porcelain crucible, a protecting crucible of Berlin porcelain is supplied, in which the crucible is heated over the open flame.

If the time of the ignition is very short, the protective crucible is unnecessary.

The porcelain and platinum boats are identical with those of the carbon and hydrogen determination.

Determination in the Crucible

• 1. The clean crucible, after igniting for five minutes, is brought to the balance on the copper block of the micro-desiccator. Platinum crucibles may be weighed after five minutes, porcelain ones after twenty minutes. Changing of the copper block, as described on p. 121, is an advantage. The crucible with lid is placed on the balance pan by means of a platinum-tipped forceps and weighed accurately to ± 0.001 mgm. The material is weighed into the crucible with a micro-spatula, using 2-5 mgm. ; this is carefully brushed down with a marten-hair brush. For weighing hygroscopic substances, the stoppered nitrogen weighing tube is used (p. 79). Oils are placed at the bottom of the crucible by means of a glass thread. The lid is replaced, the crucible put on the copper block in the micro-desiccator and placed on the large platinum lid or in the protective crucible.
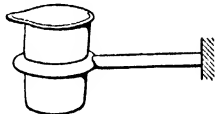
FIG. 42. — Platinum crucible in platinum wire ring, for the determination of gold, silver, and platinum. (Actual size.)

The non-luminous flame of a Bunsen burner is carefully directed on to the lid of the micro-crucible. Immediately the substance has charred, it is heated carefully underneath, with the flame gradually increased. After about five minutes the flame is turned out, the lid removed, and, if carbon is still to be seen in the crucible, 1 drop of nitric acid (specific gravity 1·4) is added from a glass thread. With very resistant substances, the addition of nitric acid must be repeated frequently.

The crucible is now placed on the copper block and weighed as before. The weight is checked, if the material is difficult to ash, by repeating the ignition with nitric acid and weighing until the weight is constant.

Even compounds containing gold and platinum may generally be analysed very accurately by direct ignition in a platinum crucible, if special precautions are taken.

The crucible and weighed material are placed in a platinum ring (Fig. 42), which is heated carefully with the normal flame of a micro-burner. First the escaping hydrochloric acid and then the products of combustion from the organic material may easily be detected by the smell. The micro-burner is then removed and the crucible heated, with a flame which is just non-luminous, for three

seconds to a dull red-heat. The lid is then removed to see whether any carbon still remains in the crucible. If so, the ignition is repeated. The results obtained are at most 0·2 per cent. below the theoretical ones.

2. *Determination as Sulphate.* The substance to be analysed, weighed in a crucible as before, is treated with 1 drop of dilute sulphuric acid (1 : 5), which is allowed to fall on to it from a capillary tube about 200 mm. long, 1–2 mm. diameter, and drawn out to a fine point. After replacing the lid, the material is heated momentarily from above, for periods of one to two seconds, with a micro-burner, so that each time very weak fumes of sulphur trioxide escape. When the sulphuric acid has evaporated, the crucible is heated underneath for three minutes with a stronger flame, to convert all the bisulphate into sulphate. The lid is then removed to see if any carbon still remains. In that case, a drop of sulphuric acid is added and the residue ignited again, repeating this until all carbon has been destroyed, and then once more. It is strongly recommended that each determination should be made in duplicate.
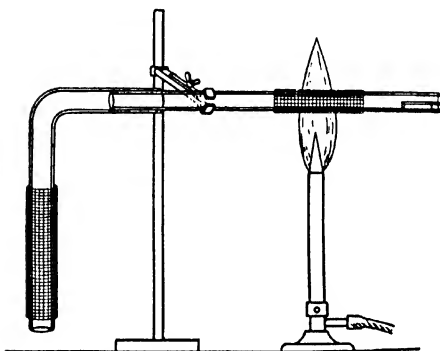
Fig. 43.—Micro-muffle.   (⅓ actual size.)

Determination in the Boat

This is carried out in the micro-muffle (Fig. 43). This consists of a Supremax tube, 200 mm. long and 10 mm. external diameter, for which purpose an old combustion tube is very suitable. This is clamped in a horizontal position at such a height above the table that it can be heated from below by the hottest part of a Bunsen burner flame. The shorter limb of a hard glass tube of 12–14 mm. inner diameter, bent at right angles, is placed over one end of the muffle tube ; it is best to insert a layer of asbestos paper between the tubes. The shorter limb of the right-angled tube is 50 mm., and the longer limb 150 mm., long. The longer limb is wrapped in a double layer of wire gauze 80 mm. long, which is heated by the flame of an inclined Bunsen burner so that an ascending air-current is induced within the tube. This attains a higher velocity in the horizontal tube in consequence of the reduced diameter. In order to enable this current of air to be uniformly heated with a second

burner, a roll of wire gauze, 50 mm. long, is passed loosely over the horizontal tube.

Course of the Analysis

The substance is weighed into a platinum or porcelain boat and treated with a drop of dilute sulphuric acid (1 : 5), which is allowed to fall from a capillary drawn to a fine point and held vertically. The boat is then inserted, by means of platinum-tipped forceps, into the open end of the horizontal tube (Fig. 43), the roll of wire gauze is placed about 30 mm. in front of the boat and heated in the middle with a Bunsen burner. The roll and burner are gradually brought nearer the boat and the reaction is observed closely. If one proceeds too rapidly the contents of the boat easily creep over the edge or even froth violently, which always results in loss of material. On proceeding carefully, very faint fumes of sulphur trioxide appear at the mouth of the tube. When the burner has arrived under the boat, the wire roll is removed and the boat strongly ignited for one minute, to convert the acid sulphate into the normal salt. If, afterwards, particles of carbon should still be visible, the boat is held by the platinum-tipped forceps and ignited rapidly once in a roaring flame, placed on the copper block and weighed shortly afterwards—the platinum boat after five, and the porcelain one after ten minutes.

A. Meixner [1] and F. Kröcker [1] found that, in spite of using the micro-muffle with care, they could not carry out the determinations with a large number of substances, because these swelled so greatly. They therefore used successfully the process already described. On the same ground, I have therefore carried out all determinations recently in the crucible only; the results are excellent. They seldom differ more than $\pm$ 0·1 per cent. from the theoretical ones.

Calculations

(a) Found : Metal.

> log (percentage of metal) = log (wt. of residue)
> $\qquad$ + 2 — log (wt. of material).

(b) Found : Metallic oxide of sulphate.

> log (percentage of metal) = log (wt. of residue)
> $\qquad$ + log F + 2 — log (wt. of material).

For F, see p. 266.

Example : 4·584 mgm. Trimethyl-heptadecabetaine-chloro-platinate yield 0·845 mgm. Pt.

[1] *Mikrochem.*, **5**, 130 (1927).

Molecular weight, $2(C_{20}H_{41}NO_2)_1H_2PtCl_6 = 1064.6$.

Platinum $\begin{cases} \text{Theory}: 18.34 \text{ per cent.} \\ \text{Found}: 18.43 \text{ per cent.} \end{cases}$

Further Methods for Determination of Metals

By direct ignition, if necessary with nitric acid, are determined :

In *Platinum Crucibles.* Iron as Fe_2O_3. Aluminium as Al_2O_3, Copper as CuO, Tin as SnO_2, Silicon as SiO_2, and Magnesium as MgO.

In *Porcelain Crucibles.* Chromium as Cr_2O_3, Silver, gold, and platinum as metal.

If the salts of noble metals have to be analysed frequently, it is advisable to keep a special crucible for them. The platinum crucible keeps its weight better than the porcelain one and the determination can be made much faster in it. When several hundreds of milligrams have been collected, the silver and gold are removed electrolytically, using the crucible as anode in the electrolysis.

A. Friedrich [1] recommends ashing the organic substances in a porcelain boat in the combustion tube in a current of hydrogen to determine cobalt and nickel as metal.

Metals estimated as sulphates are sodium, potassium, magnesium, calcium, strontium, barium, cadmium, manganese, and lead. With organic salts of lead, addition of nitric acid is essential, because the crucible is damaged if elementary lead separates. F. Pregl adds 1 drop of conc. sulphuric acid and follows it by the addition of concentrated nitric acid as long as the residue tends to darken in colour.

Lithium is evaporated with sulphuric acid in the micro-muffle. Because the sulphate is hygroscopic, it must be weighed with moisture excluded.

For further particulars of micro-determination of the metals see F. Emich, *Mikrochemisches Praktikum*, 1931, and *Lehrbuch der Mikrochemie*, published by Bergmann, Munich, 1926. Other references are :

Aluminium, chromium, iron :	K. Heller,	*Mikrochem.*	**12**, 327 (1933),
Tin, arsenic, antimony, bismuth :	K. Heller,	,,	**14**, 369 (1934),
Mercury :	F. Cucuel,	,,	**13**, 321 (1933),
Cobalt, nickel, manganese, zinc :	K. Heller,	,,	**12**, 375 (1933),
Nickel, cobalt :	Z. Stary,	,,	**15**, 140 (1934).

[1] A. Friedrich : " Die Praxis der quantitativen Mikroanalyse," p. 125.

VIII. MICRO-ELECTRO-ANALYSIS BY F. PREGL'S METHOD

General

The electrolytic determination of metals is to be used especially if the metal cannot be determined by the methods previously described or if only small amounts occur in the organic material, *e.g.*, copper in preserved vegetables.[1] Electrolysis may also be used to determine the halogens indirectly in the presence of one another ; for this the sum of the silver halides is first determined, the precipitate dissolved in 3–4 per cent. potassium cyanide solution, and the silver separated electrolytically ; the proportions of chlorine and bromine present can then be calculated.

The maintenance of the conditions, which are recognised as necessary for the quick and quantitative determination of copper on the anode, has proved to be much simpler than might have been expected in the separation of minimal amounts of copper, because it was found possible to maintain the liquid in motion by vigorous boiling instead of by the use of a stirrer. In this way the electrolysis can be carried out appreciably more quickly ; it is necessary, however, to ensure that the process is not discontinued until the liquid has cooled completely in the closed electric circuit, as otherwise the separated copper dissolves in the dilute oxygenated sulphuric acid.

Apparatus
(Fig. 44)

The cell consists of a simple test-tube, of 16 mm. external width and 105 mm. long, which is held in a ring of hard rubber by means of metal springs. The ring is clamped to the stand by a screw-clamp so that it can be adjusted laterally and vertically. The terminals of the electrodes are bent over and dipped in the small mercury contacts, *Hg*, in the rubber ring.

As cathode is used a platinum gauze electrode of cylindrical shape (Fig. 45), 10 mm. in diameter and 30 mm. long. As shown in the diagram, a stout platinum wire, which projects above for a length of 100 mm., is welded to this electrode. In order to avoid contact of the electrode with the wall of the containing vessel during its withdrawal, both upper and lower edges are each furnished with three glass beads, which are fused on to it and have a diameter of 1·5 mm. It should be noted that so-called " enamel " is not suitable for this purpose, as it dissolves appreciably on boiling the liquid during the electrolysis and thus causes negative errors.

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[1] For the determination of copper in preserved vegetables, see the second (English) edition of this book, p. 175.

A platinum wire 130 mm. long, which is bent as shown in the diagram (Fig. 45, *A*) is used as anode, *A*. To this, two Y-shaped glass extensions are sealed in order to ensure that the anode takes up a definite central position within the cathode and does not touch it during withdrawal. Both electrodes should be so arranged that they may be inserted into the electrolytic cell without touching one another.
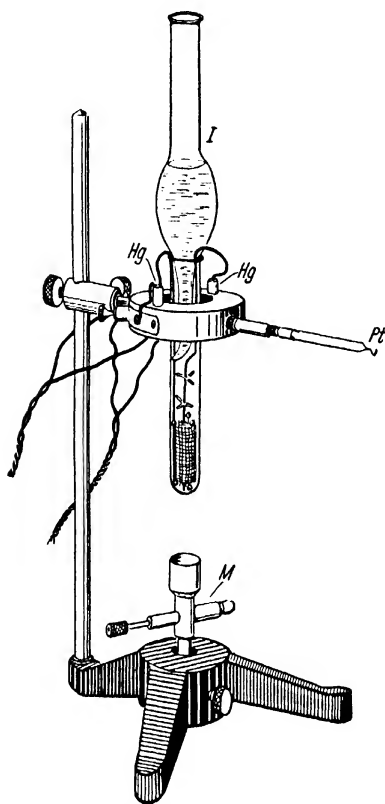
During the first experiments it was found that small losses occurred

Fig. 44.—Apparatus for the electro-analytical determination of copper. (⅓ actual size.)
I, Condenser.   *Hg*, Mercury contacts.   *Pt*, Platinum hook.   *M*, Micro-burner.

Fig. 45.—Platinum electrodes.
(⅔ actual size.)
K, Gauze cathode.   *A*, Anode.

through spirting of the liquid, or even through drops of liquid hanging to the wall of the empty portion of the cell. This fault can easily be overcome by placing in the mouth of the cell a loosely-fitting internal condenser (Fig. 44), with a laterally deflected tip. This is made from an ordinary test-tube by blowing a bulb in the middle and drawing out the closed end to a projection, about 50 mm. long, as shown in the illustration. After carefully removing any grease from its outer surface with chromic-sulphuric acid, the condenser is filled with water and used as indicated in the illustration.

As a source of current it is best to use two 2-volt accumulators, inserted in series. Into the circuit are inserted (1) an adjustable

resistance (6 ohms), (2) a commutator, and (3) a voltmeter reading up to 10 volts, as shown in the diagram of connections (Fig. 46).

The Course of the Electrolytic Determination of Copper

The organic material is decomposed by A. Friedrich's [1] methods, either in the pressure tube with conc. nitric acid (p. 105) or in the small Kjeldahl flask (p. 90) with conc. sulphuric acid, conc. nitric acid, and perhydrol. The nitric acid must always be completely driven off finally with sulphuric acid, until sulphur trioxide fumes appear. The sulphuric acid is then concentrated further on the open flame while blowing air into the flask; after this, it is diluted with water, boiled strongly for some time to drive off nitrosyl sulphuric acid and nitrous vapours, and rinsed quantitatively into the cell, which has been cleaned with hot sulphuric-chromic acid and rinsed with water. The volume of liquid should not be more than 7 c.c.

During the decomposition, everything is prepared for the analysis ; the apparatus is assembled as in Fig. 46. The platinum gauze electrode, whether covered with copper or not, is now dipped successively into concentrated nitric acid, distilled water, alcohol, and finally into pure ether and then dried about 1 mm. above a Bunsen flame for a short time (three to five seconds). The terminal of the electrode is heated for a short time in the flame to remove drops of mercury adhering to it. The electrode is cooled whilst hanging on a platinum hook, *Pt*, which is sealed into a glass rod of the micro-electrolysis apparatus.

Fig. 46. — Diagram for the electrolytic determination of copper. *E*, Cell. *V*, Voltmeter. *Akk*, Accumulators.

The electrode can be weighed after five minutes on account of the small heat capacity and high conductivity of platinum. It is weighed on the left balance pan, on which it rests on the three glass beads. First the cathode, then the anode, are introduced into the cell, and their terminals dipped into the corresponding mercury contacts. The electrolytic cell is then closed by inserting the condenser filled with cold water. Its tip should be in contact with the wall of the cell so that the condensed liquid continuously flows back. After establishing the circuit the voltage is adjusted to 2 volts by means of the resistance and the cell is heated from below with a small micro-burner. The oxygen which is evolved at the
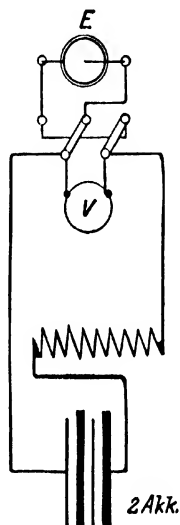
[1] A. Friedrich: "Die Praxis der quantitativen organischen Mikroanalyse," p. 128

anode prevents any superheating, and the liquid boils vigorously without bumping. *It is advisable to slip a perforated sheet of mica over the cell at the surface, in order to avoid heating of the upper portion.*

Should the voltage alter during the analysis, it must be readjusted to 2 volts by means of the resistance. In ten to twenty minutes, it may be safely assumed that the last traces of copper have been deposited on the electrode. This may be confirmed by testing with potassium ferrocyanide.

The electrolysis is completed by immersing the cell in a beaker of cold water whilst the current is maintained and using a second beaker of cold water after a few minutes. The apparatus is very convenient for this purpose, as by adjusting a single clamp the whole cell can be transferred from the flame to the cooling water whilst still in circuit. When the contents are thoroughly cooled the condenser is removed, and, after carefully washing the hands, the anode is seized with one hand and the cathode with the other, avoiding any lateral movement whilst removing them from the cell. The cathode with its copper deposit is cleaned, dried, and weighed as before. If the copper separated is again dissolved and re-precipitated, the value is easily reproducible to 0·002–0·005 mgm.

A. Benedetti-Pichler [1] has replaced the sulphuric acid in this determination of the copper by dilute nitric acid. This renders it possible to dissolve alloys in nitric acid, to dilute to the mark in a large graduated flask, and then to utilise 1/100 or 1/500 of the whole for analysis. A number of conditions must be observed for this :

1. If the concentration of the nitric acid is rather too high, a crystal of potassium sulphate must be added ; and if this is not sufficient, ammonia must be added, drop by drop, until deposition of the copper commences.

2. A somewhat higher voltage of 2·7–3·1 volts must be used.

3. A single drop of alcohol must be added at the beginning of the electrolysis, in order to avoid loss by spraying due to evolution of gas at the anode. During electrolysis the cathode should project to a height of 3–4 mm. above the liquid, in order that the walls of the cell may be washed with 1 per cent. nitric acid five minutes after starting. After a further twenty minutes electrolysis is complete. At the beginning the electrolyte is warmed until it almost boils, and the experiment is so arranged that the bath cools completely in twenty to thirty minutes. The condenser is therefore not used.

The anode is first removed, and if it is coated with a deposit of lead peroxide this is removed by placing it in a test-tube containing nitric acid to which oxalic acid has been added ; the cathode is

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[1] *Z. analyt. Chem.*, **62**, 321 (1923).

then removed and rinsed with distilled water only. Even if the copper precipitate should be spongy it adheres to the cathode if only water is used. It was found that copper which has been deposited in a spongy condition easily becomes detached and floats if the cathode is subsequently rinsed with alcohol, in consequence of surface tension. The washed electrode is dried for a short time above a Bunsen flame, and weighed after cooling for a few minutes. The analytical results which were obtained with brass filings, gun-metal, red brass filings, bearing metal, bronze filings, and metallic dust of various origins were in good agreement with the duplicate results of macroanalysis. This process shows an undoubted advantage over electrolysis in a solution of pure sulphuric acid, in so far as considerable amounts of other metal, more particularly of iron, which otherwise offer so much difficulty, do not hinder the quantitative deposition of the copper from the solution at the cathode.

The Determination of Mercury by A. Verdino's Method [1]

The determination is carried out in the apparatus described for copper (Fig. 44). It is, however, necessary to gild the platinum electrode. For this purpose, 50 mgm. of pure gold foil are dissolved in aqua regia and repeatedly evaporated to dryness on the waterbath after fresh additions of distilled water. The residue is dissolved in 5 c.c. of water, treated with 0·65 gm. of pure potassium cyanide and electrolysed at 3·5 volts at a temperature of 55° C. for two hours.

Because the so-called wet combustion has unfortunately not proved generally suitable, the organic substance is decomposed in the pressure tube. For this purpose, 3–8 mgm. are transferred from the nitrogen weighing tube with the long handle to a pressure tube, 10 drops of concentrated nitric acid (specific gravity 1·41) are added, and the material decomposed in the furnace at a temperature of 270°–280° C. for two hours. After cooling, the condensed drop of liquid in the capillary is driven out by warming and the tube opened in the usual manner after the capillary has cooled. The detached upper portion is first rinsed into the electrolytic cell, after which the contents of the tube are quantitatively introduced into the latter. The total quantity of liquid thus collected in the electrolytic cell should be about 5 c.c. Both electrodes are now introduced and electrolysis carried out for forty minutes at 3·5 volts, the cell being meanwhile immersed in a beaker filled with water which is maintained at 40° C. In order to complete the electrolysis the warm

[1] *Mikrochem.*, 6, 5 (1928).

water is replaced by cold and after a further five minutes the two electrodes are removed from the cooled electrolyte whilst the circuit is maintained. The gauze cathode is now washed in water, alcohol and ether and dried, without warming, by waving it in the air. Finally the curved support is passed quickly through a flame twice and the electrode weighed after five minutes.

In this determination it is necessary to allow for the zero point deflections of the balance as the smallest deviations affect the result very considerably. The accuracy of the method is so great, that the probable errors do not exceed ± 0.005 mgm. It must also be noted that mercury deposited in this manner can only be removed by gentle ignition of the cathode, but not completely by immersion in concentrated nitric acid.

IX. DETERMINATION OF MERCURY

Method of A. Meixner and F. Kröcker [1]

A layer of calcium oxide, 12 mm. long, is placed in an ordinary micro-combustion tube (p. 77), about 5 cm. in front of the position of attachment of the neck. This layer is held in place by asbestos
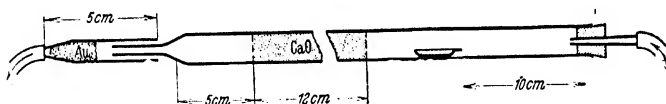
FIG. 47.—Apparatus of Meixner and Kröcker, for the determination of mercury.

plugs on both sides. The tube thus filled is ignited on a stand (p. 24) in a current of air, the velocity of which is that used in the halogen determination (p. 101). The air is taken from a steel bottle or a gasholder and led through a bubble counter. After water from any calcium hydroxide present has been driven off, the tube is further heated to redness with the tube burner. Meanwhile, from 3–8 mgm. of the substance to be analysed are weighed into a porcelain boat and this is pushed up to about 4 cm. in front of the layer of calcium oxide. A small tube filled with very fine gold wire was placed over the neck of the combustion tube (Fig. 47); the former has been weighed accurately to 0.001 mgm. The constricted end of the small tube is connected with the Mariotte flask by rubber tubing, and thus air is aspirated through this end somewhat faster than it enters the combustion tube. Hence air can be drawn in at the place where the tube passes over the combustion tube, but it cannot escape.

The substance is burnt as usual by moving a burner forward ; the

[1] *Mikrochem.*, **5**, 131 (1927).

products of combustion pass into the ignited layer of lime. Whilst mercury vapour passes through the filling and first condenses at the cold end of the tube, in front of the neck, chlorine and sulphur dioxide are retained by the lime. By means of a small flame, the mercury is driven carefully into the tube containing gold, the end of which is cooled with a small wet chamois leather. After 1 litre of air has been aspirated through with the Mariotte flask, the tube of gold is taken off, wiped, and allowed to stand for half an hour in a desiccator which is filled with calcium chloride and which has not been evacuated ; the tube is then weighed.

Simultaneous Determination of Nitrogen and Mercury by F. Hernler's Method [1]

The process depends on the fact that, in the Pregl-Dumas method for the determination of nitrogen, the mercury collects quantitatively in the cold projecting part of the combustion tube. After the removal of the azotometer, the mercury is driven into the neck of a tube containing gold, as in the previous process for mercury.

The small tube of gold is made of thin supremax glass, is 45 mm. long, and has an external diameter of 10 mm. It is somewhat blown out at one end, while at the other it is constricted and passes into a capillary 35 mm. long and of 3 mm. external diameter. The tube filling consists of a layer of tightly compressed fine gold wire, 15 mm. long.

Because carbon dioxide generated from marble and hydrochloric acid contains hydrochloric acid and therefore the formation of volatile compounds of copper must be taken into consideration, F. Hernler developed carbon dioxide in A. Schöller's [2] apparatus from potassium-sodium carbonate [3] which has been fused *in vacuo*, and dilute sulphuric acid.

For the expulsion of the mercury, which is carried out in a slow stream of carbon dioxide, fine grooves are placed in the stopcock of the gas delivery tube of the carbon dioxide apparatus as with the connecting piece, p. 78.

Course of the Analysis

Three to 7 mgm. of the substances, mixed with copper oxide, are introduced into the combustion tube as previously described. The tube is placed on the combustion stand so that 80 mm. of the end containing the neck project. So that the mercury may not enter

[1] *Mikrochem.*: Pregl-Festschrift 154 (1929).
[2] *Z. Angew. Chem.*, **34**, 586 (1921).
[3] Obtainable from Schering-Kahlbaum, Berlin.

the neck prematurely, the tube is cooled at the junction with the neck or a few centimetres before it with a moist flannel.

At the conclusion of the combustion carried out exactly as on p. 81, if micro-bubbles again rise in the azotometer the velocity of the current is adjusted at the carbon dioxide generator, by means of the grooved stopcock, to two bubbles per second. The stopcock H_2 (Fig. 22) is also opened. After taking away the azotometer the moist flannel is removed, the neck of the combustion tube wiped with a clean towel, a short roll of wire gauze placed over the end of the tube, and the tube containing gold, which has been weighed accurately to 0·001 mgm., is pushed over the neck of the combustion tube till this touches the gold. The tube of gold is clamped to a stand, its capillary connected with the Mariotte flask, by which air is aspirated through at twice the rate of the carbon dioxide, and a moistened flannel is placed around the constrictions of the tube.

Now any mercury which has distilled back is driven up to the tube burner by the movable burner, the flame of which is as small as possible ; any residues which are difficult to volatilise may be neglected. Air is then aspirated through by means of the Mariotte flask rather more strongly and the mercury is driven over into the tube of gold by heating with a very small flame while moving the roll of wire gauze slowly forward.

When the burner has quite reached the tube of gold, it is allowed to remain there and the mercury is driven over into the tube with a micro-burner. To remove the last traces, the tube is heated once more with the burner and finally with the micro-burner. The flame is always so small that glowing of the roll of wire gauze is not observed.

Afterwards, suction with the Mariotte flask is increased. After about $1\frac{1}{2}$ litres of air have been drawn through, the tube of gold is wiped first with a moist flannel, then with chamois leather, placed on the balance pan and weighed after twenty to thirty minutes.

The method is accurate to $\pm$ 0·5 per cent. The mercury is removed from the gold before each determination by warming gently in the current of air.

C. DETERMINATION OF THE GENERAL GROUPS

I. F. Pregl's Method for the Microanalytical Determination of Carboxyl

History, Principles, and Experiments

F. Pregl's wide experience with the titration of ammonia by the micro-Kjeldahl method, as well as with the preparation and pre-servation of the N/100 titration solutions, form the basis for the

determination of the equivalent weight. His device of repressing the hydrolysis of the sodium salts of weak acids by means of 50 per cent. alcohol permits of the determination of equivalent weights up to about 600 on a normal micro-weighing, with great accuracy ($\pm$ 0·5 per cent.).

Slightly soluble carboxylic acids—bile acids, saponins, etc.— are dissolved by boiling in pure methyl or ethyl alcohol which has been distilled from solid caustic potash and are titrated after dilution with CO_2-free water. Substances which are reprecipitated even on the addition of a few drops of water cannot be titrated unless they go into solution again as alkali salts. Tests introducing pyridine or acetone as solvent have been a failure because the sharpness of end-point required is unattainable. F. Pregl's directions, to use 50 per cent. alcohol, are to be followed as convenient. The expert can go up to a concentration of 65 per cent. of alcohol if necessary, because in these circumstances the end-point can still be clearly observed with 1 drop (0·02 c.c.). If the concentration of alcohol is raised still more, the end-point is ascertainable with certainty only to 2 or 3 drops. For colourless substances, besides phenolphthalein every indicator which gives the required sharp end-point may be used. Thymolphthalein, with an end-point interval 9·3–10·5 p_H, is very suitable for the titration of substances coloured yellow to red ; the sharpness of the end-point, from colourless to the first bluish-green, is not inferior, in daylight, to that of phenolphthalein. Very deeply coloured substances are only determined by titration if the formation of their sodium salts is linked with a marked loss of, or change in, colour. If supplied with apparatus for electrometric titration, one should here give preference to this method.

Besides organic acids, lactones may also be determined quantitatively by titration. In some cases, lactones are only hydrolysed by long boiling with excess of alkali, in other cases, they react as readily as organic acids. The active hydrogen must be determined in order to decide whether a lactone or a free carboxylic acid is present. In special cases, the titration with alkali also enables us to determine phenolic hydroxyl groups, as in picric acid, and enolic hydroxyl groups, as in ascorbic acid, vitamin C. If ascorbic acid is boiled with excess of alkali, a larger consumption of alkali occurs, which is due to the partial splitting of the lactone ring. In the cold, and even in warm aqueous alcohol, on the other hand, one of the two enolic hydroxyl groups can be determined accurately. Amino acids are determined, according to the method of W. Grassman and W. Heyde,[1] by titration with alcoholic sodium hydroxide.

[1] *Z. physiol. Chem.*, **183**, 32 (1929).

Especially with natural products of unknown constitution it appears essential to check the consumption of alkali in the cold, warm, and also after boiling with excess of alkali.

The Microburette

The burettes, of 10 c.c. capacity, are divided into twentieths of a cubic centimetre and provided with Schellbach scales. Recently, the firm of P. Haack, Vienna, has supplied microburettes of Jena glass (Fig. 48). They are preferable to the ordinary glass burettes in that they yield no alkali after years of use, so that the volumetric solutions are stable permanently. The reading is taken with an adjustable lens. The burettes are kept in a titration stand, which consists of a metal stand with two horizontal " Eternit " plates arranged one above the other. A horizontal and a vertical pane of opaque white glass are placed in front, which facilitate the recognition of the end-point of the titration. For the determination, the solution to be titrated is pumped into the burette from the storing vessel of 500 c.c. capacity. The solution enters the burette through a glass tube placed behind it, which ends in a capillary. The capillary ends exactly at the zero and immediately the pressure is released the solution standing above the mark is automatically syphoned back again into the storing vessel. The solutions are protected from carbon dioxide from the air by means of small tubes of soda-lime or ascarite.
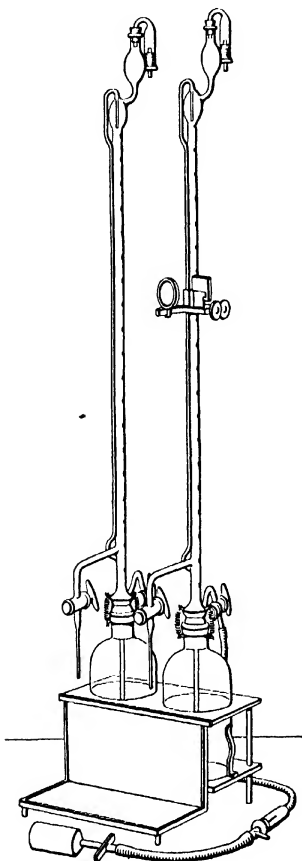
FIG. 48.—Microburettes in the titration stand, with device for filling and automatic zero standardisation, by the firm of P. Haack, Vienna.

In order to have solutions free from carbon dioxide, always in stock, the filling of these tubes must be frequently renewed.

F. Pregl, in the second (English) edition of this book, p. 115, recommends that pieces of rubber tubing should connect the burettes

with the capillary outlets, and that the withdrawal of the solution should be carried out with a pinch-cock or by pressure on a small glass sphere in the bore of the rubber tubing. Glass cocks placed obliquely, which have been fused on, are preferred by A. Friedrich.[1] Both methods permit 0·01 c.c. to be withdrawn without difficulty after some practice. From experience, the removal of alkali by means of glass spheres may here be recommended. In order that, on the one hand, standard solutions may be removed rapidly, and, on the other, the smallest drops may be withdrawn with the greatest accuracy, the capillary outlet is 6-8 cm. long, 0·3 cm. bore, and is narrowed to about 1 mm. in the lowest part only. Thick-walled capillaries are to be drawn out thin on account of the smaller risk of breaking and easier manipulation. They are ground conically at the outlet. Small droplets of liquid form a very visible border at the rough flat edge and may be removed by contact with the surface of the liquid to be titrated.

The Standard Solutions

In recent years F. Pregl had given up the N/40 solution originally recommended for the determination of the carboxyl group and the N/70 solution used for the titration of ammonia by the Kjeldahl method, which made a direct calculation of the results possible, and had carried out all microtitrations with N/100 solutions for the sake of uniformity.

N/100 *Hydrochloric Acid.* Fifty c.c. of N/10 HCl, which has previously been most accurately standardised against sodium carbonate, are diluted to 500 c.c. with boiled water, free from carbon dioxide, in a measuring flask, placed in the clean dry vessel for the burette described above, and checked with pure sodium carbonate, using 3-4 mgm. If the acid has been carefully prepared, the factor does not alter even on standing for several months.

N/100 *NaOH* is prepared by F. Pregl's " approximation method "[2] from Sörensen's " oily lye," which is prepared most simply and rapidly by dissolving tablets of pure sodium hydroxide in an equal weight of water with constant shaking in a bottle, closed with a rubber stopper, which becomes very hot. It is then placed in a pan of water at about 80° C. On account of the decrease in viscosity at the high temperature, the insoluble sodium carbonate often settles within half a day.

Into the 500 c.c. supply bottle for the burette, 400 c.c. of boiled-out distilled water are introduced, about 0·3 c.c. of the clear " oily lye " are then added ; the bottle is closed with a cork and shaken. Of this solution, 5 c.c. are pipetted out and titrated with the pre-

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[1] A. Friedrich : "Die Praxis der quant. org. Mikroanalyse," p. 173.
[2] *Z. analyt. Chem.*, **67**, 23 (1925).

viously prepared N/100 hydrochloric acid, using phenolphthalein as indicator, the liquid being boiled up thoroughly before the end-point is reached. The calculated divergence is then corrected to within 10 per cent. by the addition of boiled-out distilled water and the process repeated. The final correction is made after twenty-four hours only, using the burette belonging to the supply bottle. For this, the potassium bi-iodate recommended by M. K. Zacherl and H. G. Krainik [1] is weighed into a quartz flask. The bi-iodate, $KH(IO_3)_2$, prepared according to the method of M. König and I. M. Kolthoff,[2] is readily obtained analytically pure and is stable indefinitely. Owing to its high molecular weight, very accurate determinations are possible.

Because 1 c.c. of N/100 solution contains 3·8996 mgm. of potassium bi-iodate, the weighed amount of iodate divided by 3·8996 gives the amount of N/100 alkali theoretically required for the titration.[3] Under the conditions of titration to be described later, non-volatile organic acids are also suitable for the determination of the factor as well as for the later checking of the alkali. Phenolphthalein or thymolphthalein, but not methyl orange, may be used as indicators. Thus, benzoic acid is used for the standardisation of very dilute solutions of alkali for the determination of acetyl and benzoyl groups. If the weight of benzoic acid taken is divided by 1·2205, the theoretical number of cubic centimetres of N/100 alkali required for the titration is obtained.

Phenolphthalein Solution: 1 gm. of phenolphthalein solution is dissolved in 100 gm. of 96 per cent. alcohol.

Thymolphthalein Solution: 0·1 gm. of solid is dissolved in 100 gm. of 96 per cent. alcohol.

Course of the Analysis

Three to 9 mgm. of the substance to be analysed are weighed into a cleaned 100 c.c. quartz flask,[4] from the long-handled weighing tube used for the nitrogen determination. If the substance is not easily soluble, it is first finely powdered in an agate mortar. Oily and volatile substances are weighed in small boats [5] or micro-beakers.[6]

Titration by Pregl's Method. To 10 c.c. of pure absolute alcohol and 10 c.c. of water, 2 drops of phenolphthalein are added; the mixture is heated to the boiling point and titrated with N/100–NaOH until a pink coloration is just produced. It is then decolorised with

[1] *Mikrochem.*, **11**, 61 (1933).
[2] I. M. Kolthoff : " Die Massanalyse," II., p. 106. (Berlin, 1928.) (Julius Springer.)
[3] The potassium bi-iodate is also used for the determination of the factor for the sodium thiosulphate solution.
[4] Or in a small steamed-out Jena glass Erlenmeyer flask.
[5] Determination of carbon and hydrogen, p. 49.
[6] Micro-Carius method, p. 106.

0·1 c.c. of N/100–HCl, boiled for half a minute and then neutralised whilst at the boiling point with N/100–NaOH until a faint pink coloration is again produced. Two to 4 c.c. of this neutral 50 per cent. alcohol are added to the weighed substance in the quartz flask, the liquid warmed if necessary, and titrated with N/100–NaOH until a pink colour is just produced, after which 0·1 c.c. of N/100–HCl is immediately added and the liquid boiled for half a minute to drive out all carbon dioxide. The liquid is then immediately titrated with N/100–NaOH till the first traces of a pink coloration occur, which should endure for several seconds at least. It is easy to convince oneself that 0·01 c.c. of N/100–NaOH produces a definite change from a colourless condition to one in which a pink colour persists for several seconds. This determination is found difficult by beginners, particularly by those relatively insensitive to colour. Workers with a normal sense of colour carry out the determination quite correctly after a few failures.

Direct Titration.[1] •Even in the second (German) edition of this book, F. Pregl emphasised the fact that correct and freshly prepared solutions used up the same volumes on titration in the cold as after boiling-up with acid. I therefore prefer to protect the prepared solutions and solvents most carefully from carbon dioxide and to carry out the titration directly with alkali, letting the drops follow one another quickly as in the acetyl determination. Recently, at the Kaiser-Wilhelm-Institut, determinations carried out in this way have enabled equivalent weights to be ascertained with Pregl's accuracy. Only one titration is required and the addition of acid on account of the error in reading is avoided. The decolorisation which gradually appears at the drop run in enables the approach of the end-point to be noted and only carelessness makes it necessary to titrate back with N/100 acid.

About 20 c.c. of methyl or ethyl alcohol for the repression of the hydrolysis are boiled for ten minutes with 2 gm. of solid potassium hydroxide under a reflux condenser in a steamed-out 100 c.c. Erlenmeyer flask, which is protected from atmospheric carbon dioxide by a soda-lime tube ; the alcohol is distilled off and kept away from carbon dioxide. This alcohol is stable for two to three days ; when mixed with the same amount of boiled-out water under the conditions of analysis it gives a definite pink coloration on the addition of 0·02 c.c. of N/100–NaOH, with phenolphthalein as indicator. The water necessary for the determination is freed from carbon dioxide by keeping it boiling gently for some time over a small flame.[2]

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[1] Unpublished.
[2] If titrated cold, the flask is closed with a soda-lime tube and cooled in a stream of tap-water.

Into the weighed substance in the quartz flask, 3 c.c. of alcohol are pipetted and the flask closed immediately. To dissolve the substance, the flask is waved about over a small flame and just boiled, if necessary. If the substance has not dissolved completely in ten seconds, one attempts to dissolve it by adding a further 1–2 c.c. of alcohol and again boiling up. If it only dissolves partially even then, a second sample is taken and pulverised extremely finely in an agate mortar. Certain organic acids, *e.g.*, saponins, only dissolve after long boiling. In this case it is found advisable to note the time from the beginning of the boiling until the dissolution. With a test substance, the weight of which is to be chosen so that the alkali used is equal to that of the actual analysis, to ± 0·2 c.c., the excess of alkali used is ascertained under the same conditions and subtracted in calculating the result of the analysis.

The flame is now removed from the boiling water, and 3 c.c. are withdrawn in a pipette and allowed to flow into the flask containing the dissolved substance. The flame is then placed again under wire gauze, the water again kept boiling gently and the titration carried out immediately. One ascertains that the burette is filled up to the zero, adds 2–3 drops of indicator to the solution, and allows several drops of alkali to run in at first in quick succession, whilst shaking round and continuously observing the decolorisation above described. As the end-point is approached, only half drops (0·01–0·02 c.c.) are withdrawn by slight pressure on the upper half of the sphere. If a pink colour appears, the tip of the capillary outlet is touched with the surface of the liquid to be titrated, to remove the alkali still adhering to it, and the solution shaken round. If the colour still remains after three seconds, the titration is complete. After emptying the flask and rinsing with water, it is used for the next determination.

Calculation

$$\text{Equivalent weight} = \frac{\text{mgm. of substance} \times 100}{\text{c.c. } N/100 \text{ NaOH}}$$

$$\log (\text{equivalent weight}) = \log (\text{mgm. of substance}) + 2 - \log (\text{c.c. } N/100 - \text{NaOH}).$$

Examples of the Direct Titration :

Cholic acid, $C_{24}H_{40}O_5 + \frac{1}{2}H_2O$.

8·496 mgm. are equivalent to 2·04 c.c. of $N/100$–NaOH.

Equivalent $\begin{cases} \text{Theory, } 417\cdot34. \\ \text{Found, } 416. \end{cases}$

Mannonic acid, $C_6H_{10}O_6$.

(a) 8·702 mgm. are equivalent to 4·92 c.c. of N/100–NaOH.

(b) 8·815 mgm. are equivalent to 5·01 c.c. of N/100–NaOH.

Equivalent {Theory, 178·08.
{Found, 177; 176.

Picric acid, $C_6H_3O_7N_3$. (Indicator, thymolphthalein.)

(a) 8·533 mgm. are equivalent to 3·71 c.c. of N/100–NaOH.

(b) 8·632 mgm. are equivalent to 3·87 c.c. of N/100–NaOH.

Equivalent {Theory, 229·05.
{Found, 230; 238.

Ascorbic acid, $C_6H_8O_6$.

8·385 mgm. are equivalent to 4·72 c.c. of N/100–NaOH.

8·534 mgm. are equivalent to 4·84 c.c. of N/100–NaOH.

Equivalent {Theory, 176·06.
{Found, 177; 176.

II.   The Titration of Amino Acids, by the Method of W. Grassman and W. Heyde [1]

The method of R. Willestätter and E. Waldschmidt-Leitz,[2] for the determination of the carboxyl groups of amino acids by titration with alkali in alcoholic solution, has been developed as a micro-method by the authors. The titrations are carried out in an N/100 solution of sodium hydroxide in 90 per cent. alcohol. Thymolphthalein, recommended as indicator by E. Waldschmidt-Leitz is also very satisfactory for micro-titration. The first pale blue colour, not the first colour change, is taken as end-point. It has been found necessary to carry out the titrations to a colour standard because the end-point is not sharp. An M/400 solution of cupric chloride in excess of ammonia is used for comparison.

It is best to titrate in a box lined white inside, by the light of a 200-candle power daylight lamp screened from other light. To obtain the colour required, more than the theoretical amount of alkali is used up, which depends on the amount of alcohol used. Since it makes no difference, for a given volume of alcohol, whether an amino acid or a mineral acid is titrated, to complete the experiment one has to carry out a blank test with the amount of alcohol corresponding to that added to the test, and the amount of N/100–NaOH added must be subtracted from the volume found in the analysis.

[1] *Z. physiol Chem.*, **183**, 32 (1929).

[2] *Ber. dtsch. chem. Ges.*, **54**, 2988 (1921). Also, E. Waldschmidt-Leitz: *Z. physiol. Chem.*, **132**, 181 and 192 (1923/24).

Course of the Analysis

The substance to be analysed is weighed into a graduated 1 c.c. flask, which has a ground-glass stopper, and dissolved. A measured volume, e.g., 0·2 c.c., is transferred to a small flask by means of a fine capillary pipette, subdivided into thousandths of a cubic centimetre ; 2 drops of an alcoholic 0·1 per cent. solution of thymolphthalein are added and the N/100 alkali is run in until the pale blue colour is obtained. Then absolute alcohol, of nine times the volume of the original solution, e.g., 1·8 c.c., is added with a pipette, when the blue colour disappears. The titration is finished by adding fresh alkali up to a definite pale-blue coloration.

This method yields very accurate results ; it is very suitable for the determination of small amounts of amino acids, if these are not in extremely dilute solution. With N/100 solutions, the amount of alcohol to be added is ten times the volume of the solution taken for analysis. If the amino acid solution is more dilute, the concentration of alcohol may be reduced down to 80 per cent. According to L. J. Harries,[1] this is sufficient practically to repress the hydrolysis of the alkaline salts of amino acids.

III. Determination of the Active Hydrogen, by H. Roth's Method [2]

History, Principle, and Summary

L. Tschugaeff [3] observed in 1902 that the magnesium alkyl halides of lower molecular weight react with carboxyl and hydroxyl groups, with evolution of gas. His pupil, Th. Zerewitinoff,[4] afterwards pointed out that the corresponding hydrocarbon is thereby formed, and used this reaction for the quantitative determination of active hydrogen. Zerewitinoff himself, as also H. Hibbert and J. J. Sudborough,[5] have already pointed out experimental difficulties which depend on the great capacity of the magnesium methyl iodide for reaction with oxygen and water. B. Flaschenträger [6] was the first to undertake the working out of a Zerewitinoff micromethod, by which the Grignard reaction was carried out in an atmosphere of air.

The substance, dissolved in an inert solvent, is treated with

[1] *Proc. Roy. Soc. B*, **95,** 500 (1923).
[2] *Mikrochem.*, **11,** 140 (1932) ; partly unpublished.
[3] *Ber. dtsch. chem. Ges.*, **35,** 3912 (1902).
[4] *Ber. dtsch. chem. Ges.*, **40,** 2033 (1907) ; **41,** 2233 (1908) ; **42,** 4802 (1909) ; **43,** 3590 (1910) ; **47,** 1659, 2417 (1914) ; also, B. Oddo : *Ber. dtsch. chem. Ges.*, **44,** 2048 (1911).
[5] *Proc. Chem. Soc.*, **19,** 285 (1903) ; **20,** 165 (1904).
[6] *Z. physiol. Chem.*, **146,** 219 (1925).

magnesium methyl iodide and the liberated methane collected in a burette.

$$RH + CH_3MgI \rightarrow RMgI + CH_4.$$
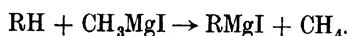
In order to produce a method which on the one hand rejects a lucky compensation of opposite errors and on the other hand needs no empirical correction, with the transference of Zerewitinoff's principle to micro-dimensions the extremely unpleasant properties of the Grignard reagent must be specially taken into consideration. According to exhaustive investigations by J. Meisenheimer and W. Schlichenmayer [1] on the sensitivity of magnesium methyl iodide to oxygen, it is essential to work in an indifferent atmosphere. It is most convenient to use nitrogen for this, as P. M. and G. F. Marrian [2] have already done with their Zerewitinoff micro-method. Very pure nitrogen, containing less than 0·2 per cent. of oxygen, is easily prepared from nitrogen from steel bottles. With this, a source of error which produces a decrease in volume is completely eliminated.

A further condition is the complete absence of moisture in the apparatus and reagents. The necessary instructions on the drying of the apparatus and the nitrogen will follow later.

Even with careful removal of the oxygen and water, the micro modification of the method is still subject to an error which depends only on the amount of Grignard reagent. The methyl iodide which still remained, to a small extent, in the Grignard reagent was recognised as the source of the small blank value, which increases considerably on warming the reaction flask above 50°. It has been shown, however, that the slightest traces of methyl iodide may be removed in an atmosphere of nitrogen under diminished pressure at 50° C. ; the reagent thus pre-treated has proved quite satisfactory.

It is self-evident that the reaction can only be quantitative if the substance analysed is entirely dissolved. Besides, the few solvents which can be considered—isoamyl ether, xylene, anisole, and pyridine—must be of such a degree of purity and dryness that they permit absolutely no evolution of gas to be detected in the blank test. Of these, anisole, and, still more, pyridine are suitable on account of their low vapour pressure and good solvent power. The usual drying of the anisole over sodium was very soon proved insufficient. If, however, anisole is shaken round with phosphorus pentoxide and kept over it, the last traces of moisture are removed. Because many substances can only be dissolved in anisole on warming or even on heating to the boiling point, it has often been found advisable to dissolve the substance in a mixture of a few drops of

[1] *Ber. dtsch. chem. Ges.*, **61**, 2029 (1928).
[2] *Biochem. J.*, **24**, 746 (1930).

pyridine and anisole and to subtract the value of the blank test, due to the pyridine, from the volume read.

Recently, pyridine purified over perchlorate and carefully dried has been prepared ; this answers all requirements and shows no evolution of gas in blank tests on heating up to 50° C. If it is necessary to carry out the reaction at temperatures above 50° C., the volume of gas (0·05–0·08 c.c.) found in the blank test at the same temperature must be considered. Because pyridine is a con- siderably better solvent than anisole, the method has now obtained a wider range of application, *e.g.*, for sugars, many sterols, etc., which dissolve very readily in pyridine or go into solution on warming gently. A further advantage is offered by pyridine in the dissolution of substances which undergo decomposition on warming with anisole ; this can occur with many natural products, *e.g.*, alkannin.

In pyridine, the Grignard reaction is complete at room tempera- ture with almost all substances, while with anisole, particularly in the presence of several active hydrogen atoms, *e.g.*, in cholic acid, the reaction is only complete at 95° C.

In pyridine at room temperature, one atom of hydrogen in hydroxyl, sulphohydryl and carboxyl groups, two in water of crystallisation, are active ; one hydrogen atom of acetamide reacts in the cold, two at 95° C. ; in urea, two react in the cold and 3·5–3·8 at 95° C.[1] ; aniline has one active hydrogen, both in the cold and on warming ; *o*-phenylene-diamine has two in both cases ; acetanilide has one active in the cold.

With azo-benzene, the evolution of ethane may be determined either at room temperature or after heating to 95° C.

If nitro or sulpho groups occur in the structure of the ring, as in picric acid and sulpho-salicylic acid, these are not affected by the Grignard reagent at room temperatures ; on warming, however, evolution of gas occurs which makes the results useless.[2] If sulphosalicylic acid must be warmed for dissolution in anisole, too much gas is liberated by the Grignard reagent even after cooling to room temperature.

The halogen atoms in *o*-chlorobenzoic acid, *m*-bromobenzoic acid, and *p*-iodo-benzoic acid are not affected by the Grignard reagent either hot or cold, one molecule of methane is formed from the carboxyl group only.

Reference may be made to an interesting observation on an unsaturated lactone, carolic acid, analysed incidentally for Prof. H.

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[1] Time of reaction : five to eight minutes.

[2] *Cf.* J. Houben and H. Weyl : " " Methoden der organischen Chemie," Vol. 4, p. 201, 1928.

Raistrick.[1] In anisole, as was to be expected, the Grignard reaction was negative ; in pyridine, one molecule of methane was obtained. The authors explain this as an enolic transformation with the use of pyridine as solvent. In view of this phenomenon it is advisable to keep anisole in stock as well as pyridine.

W. Lüttgens and E. Negelein[2] have shown that, with carefully prepared reagents, the method permits of the determination of active hydrogen even with extremely small amounts of substance. They checked the method, using anisole as solvent, by the manometric experimental technique of O. Warburg, and obtained excellent results even on weights of 0·9 mgm.

For an analysis without heating, with some experience twenty-five to thirty minutes are required ; the accuracy is to $\pm$ 3 per cent. of the theoretical.

*   **Apparatus**

The Jena glass vessel (Fig. 49) for the reaction has a capacity of about 17 c.c. and consists of the two limbs A and B. Absolute air-tightness is attained by means of a ground-in joint 25 mm. long, which is secured with two steel springs. The nitrogen inlet tube, of 2 mm. bore, and the delivery tube for nitrogen or methane, 1 mm. bore, are sealed into the hollow stopper. The cock H_a serves to close the system after the displacement of air. The inlet tube is forked at the end ; rapid displacement of the

FIG. 49.—Apparatus for the determination of active hydrogen.

air from A and B is thus possible. The prongs are exactly in the middle of the limbs and parallel to them. With too deep a position, the screwing of the stopper into the vessel is hindered ; besides, the Grignard reagent would flow into the fork on tilting the apparatus.
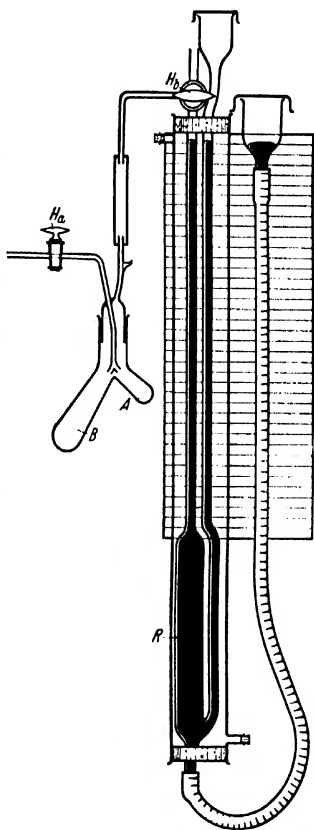
[1] P. W. Clutterbuck, H. Raistrick, and F. Reuter : *J. of Biochem.*, **29**, 300 (1935).
[2] *Biochem. Z.*, **269**, 177 (1934).

The required air-tightness of the inlet tube is obtained through connections of glass in contact with glass, at which pressure-tubing treated *in vacuo* with paraffin is used.

The Grignard reaction is carried out in the limb *B*, the long shape of which prevents moistening of the stopper whilst shaking. The short limb *A* serves for the reception of the Grignard solution. With the vessel in the vertical position it holds 2·5 c.c. of reagent. An inclination of about 60 degrees is sufficient to bring all the Grignard reagent into reaction with the substance in limb *B*.

The microburette is connected with the reaction vessel by a small side tube bent downwards at right angles and by a piece of absorption tubing (p. 26) which is 70 mm. long. The nitrogen or methane enters the burette through the three-way cock (or a T-piece with stop-cocks in it). The burette is 37 cm. long, graduated in hundredths of a cubic centimetre, and has a capacity of 4 c.c. For reactions at higher temperatures the reaction vessel must in the meantime be warmed up to 95° C. In order that the excess pressure thus set up may be reduced by lowering the level of the mercury, a gas or mercury reservoir *R*, of 10 c.c. capacity, is sealed on at the end of the burette ; from this, the limb for the comparison branches off at the lowest point. Mercury is used as sealing liquid. A cylinder filled with water surrounds the entire burette.

For reading the volume of gas the three levels of mercury are brought to the same height ; a lined card placed behind the burette facilitates the estimation to the thousandth part of a cubic centimetre.

Because the volume of methane read is very small in proportion to the volume of the reaction vessel, the readings of the mercury must be made at exactly the same temperature before and after the determination. In order to carry out the readings always at the same temperature (room temperature), a container holding 20–30 litres of water is kept in the laboratory. Water is drawn off from this in a litre beaker for cooling the reaction vessel before and after the analysis. For reactions at higher temperature, corresponding water-baths serve.

Reagents

Purification and Drying of Nitrogen

Because steel bottles with nitrogen of the highest degree of purity are not always at hand in the laboratory, the necessary purification of commercial nitrogen will next be described.

Commercial Nitrogen with up to 5 *per cent. of Oxygen.* To avoid repeated reducing of the copper oxide formed and to permit the gas to remain in contact with the copper as long as possible, an

ordinary combustion tube, of 23 mm. bore, is used. This is charged
with pieces of reduced copper wire to a length of 50 cm. ; two layers
of wire-form copper oxide, 5 cm. long, which are held in place by
two spirals of wire gauze, enclose the copper on both sides. The
tube thus filled is heated to dull-red heat by a combustion furnace.
For further purification, the gas is led through a wash-bottle
,containing 50 per cent. alkali and is then dried by passing through
a similar one containing concentrated sulphuric acid. The last
traces of moisture are retained in a U-tube filled with phosphorus
pentoxide.

Nitrogen, of 99·5 *per cent. purity.* A macro-combustion tube is
cut down to a length of 35 cm. and filled with a layer, 15 cm. long,
of pieces of reduced copper wire, and with two short copper spirals
which enclose this layer. The tube filling is kept glowing on a
micro-combustion stand by means of a micro-burner. The further
purification and drying are as above.

Nitrogen, of 99·9 *per cent. purity,* which is used for filling electric
bulbs, can be obtained from the Osram G.m.b.H., Berlin. Purifica-
tion of this is found to be unnecessary. The nitrogen is led from
the bomb immediately through the drying arrangement into the
reaction vessel.

To store the purified nitrogen in a gasholder has been found
impracticable. The nitrogen taken from the gasholder contains
oxygen from the air even after a few days, which originates in the
water which seals the nitrogen.

After completion of the analysis the apparatus for purifying and
drying the nitrogen is to be kept and protected against the penetra-
tion of moisture by sealing with a small guard tube of phosphorus
pentoxide.

Purification of the Reagents

Isoamyl-ether is allowed to stand for a few days over sodium
and afterwards distilled from fresh sodium (b.p. 171°–172° C., at
750 mm.).

Magnesium is used in ribbon form. Pieces of ribbon 30 mm.
long are cleaned with dilute acetic acid, alcohol, and ether. In the
preparation of the Grignard reagent, the commercial shreds yield a
fine sludge, which obstructs the pores of the fritted glass plates
during suction ; its use has therefore been given up.

Methyl Iodide. Commercial methyl iodide may be purified by
fractional distillation (b.p. 43° C.) and then serves the same purpose
as Merck's analytical reagent.

Anisole is distilled from sodium and kept over this (b.p. 152·5°–
153° C. at 761 mm.).

Pyridine. Five hundred grams of pyridine are freed [1] from homologues over perchlorate according to the directions of F. Arndt and P. Nachtwey.[2] The pyridine which has been distilled *in vacuo* is mechanically shaken for six hours with several pieces of barium oxide in a strong bottle with ground-in stopper. If solid barium oxide is still present [3] after shaking, the pyridine is filtered quickly through a folded filter into a bottle with a cap, in which coarse, barium oxide is placed. After a few days the pyridine is ready for use.

Preparation of the Grignard Reagent

Fifty grams of isoamyl-ether, 4·5 gm. of cleaned magnesium ribbon, and 18 gm. of methyl iodide are placed in a dry 150 c.c. round-bottomed flask, which is combined with a ground-in reflux condenser with a calcium chloride tube. A few crystals of iodine may be added to start the reaction. The flask, with the condenser, is then placed on an unheated water-bath and heating is started. The reaction thus occurs gradually ; if it is too vigorous the water-bath is removed. From the beginning of the reaction the water-bath is heated to boiling for half an hour and then removed.

After cooling to about 30° C. the reflux condenser is replaced by a nitrogen inlet tube and a ground-in descending condenser. The excess of methyl iodide is now distilled off for half an hour on the water-bath whilst passing in nitrogen. Meanwhile a glass suction filter (11 G 4 Schott) and a 100 c.c. filter flask are dried in the oven at 110° C. The hot flask, with the filter attached, is connected with a good water-pump and nitrogen is drawn through the filter from the container. If the flask and filter were allowed to cool in air after removal from the oven, a coating of moisture would be formed, which would react with the reagent. After the Grignard reagent and filter have cooled to room temperature, the turbid reagent is decanted from the residue of magnesium ribbon into the filter. The suction may occasionally last from half an hour to one hour. Unnecessary admission of air is avoided, if the tubing is removed from the pump whilst 1–2 mm. of Grignard solution still remain on the filter. Notwithstanding the fine film formed by shutting off the vacuum, the clear solution is decanted into an absolutely dry 100 c.c. flask, with ground-in stopper, which has been cooled whilst nitrogen is passed through it.

This reagent still contains traces of methyl iodide, which must be removed. For this purpose a Claisen flask, of 100 c.c. capacity,

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[1] This purified pyridine may be obtained from Dr. Fraenkel and Dr. Landau, Berlin-Oberschönweide.

[2] *Ber. dtsch. chem. Ges.*, **59,** 448 (1926).

[3] Otherwise, fresh barium oxide is added and the shaking repeated for the same time.

is alternately evacuated and filled with nitrogen ; the filtrate is then brought into the flask and evacuated. Sudden breaks in the vacuum are avoided by means of pinchcocks ; a manometer is inserted as a check. The remaining traces of methyl iodide are now removed in an atmosphere of nitrogen under diminished pressure on a water-bath at 50° C. for half an hour. Whilst the Grignard reagent is cooling, the flask is slowly filled with nitrogen from the bomb, using a mercury excess pressure valve.

The reagent which was formerly decanted into small flasks was more or less stable. For a long time the sealing of the reagent under nitrogen in bulbs has been strongly approved. For this, ten to twelve test-tubes are dried in the drying-oven at 110° C., cooled in an atmosphere of nitrogen, and closed with tight-fitting rubber stoppers. The Grignard reagent is quickly decanted into these tubes, in portions of from 3 to 7 c.c., through a small funnel which ends 6 cm. above the bottom of the test-tube. The test-tubes are now sealed with the blowpipe 5–6 cm. above the Grignard reagent and allowed to cool slowly. Sometimes black rings are formed below the place where the tube is sealed, which have no bad effect, because on opening the bulb is burst immediately above the reagent. The reagent is perfectly clear and has a yellowish-green tinge. It is permanently stable. The solution is 1·2–1·4 N. Its strength is determined by decomposing 1 c.c. with excess of N/10–HCl and titrating back with N/10 alkali, using phenolphthalein as indicator.

Preliminary Treatment

Complete solubility in pyridine or anisole of the substance to be analysed and absence of moisture are most important for a satisfactory determination. All substances must be most finely powdered and dried in a desiccator over phosphorus pentoxide for several hours. Occasionally alcohol of crystallisation and water of crystallisation are strongly retained by the substance and only given up slowly, so that drying at 100° C. and even above it is indispensable.

If anisole is used as solvent, the amount required for the determination is placed in a small bottle with ground-in stopper, mixed with about one-twentieth of its weight of phosphorus pentoxide, and shaken well several times. On standing the phosphorus pentoxide settles at the bottom and the clear anisole may be pipetted off for the determination.

A Grignard bulb is filed about 1 cm. above the liquid and the tip broken off with a hot glass drop. The contents are poured into a small flask with ground-in stopper, which has been dried in the drying-oven at 115° C. and cooled whilst nitrogen is passed through and the stopper is firmly inserted.

Course of the Analysis

As is usual in volumetric determinations, the amount of substance weighed corresponds to the volume of gas required.

If solid the substance is weighed, from the desiccator, into the small weighing tubes with long handles and placed at the bottom of limb *B*. If the substance has been quantitatively dried in a small platinum boat, this is allowed to slide into the limb *B* by holding the reaction vessel obliquely. For weighing oils and liquids, the micro-weighing bottles (p. 106) are best used. Liquids with high vapour pressure are weighed in J. Pirsch's capillary, and this is pushed, with the tip downwards, into limb *B*. For this, the solvent has been previously placed in the reaction vessel. By carefully warming in a stream of nitrogen the liquid is driven out of the capillary.

The reaction vessel, cleaned with acidified water, water, alcohol, and acetone, the stopper, and the pipettes required are placed in the drying oven at 110° C. The reducing valve is adjusted so that 100–150 c.c. of nitrogen stream out of the bomb in five minutes.

The reaction vessel is then removed from the oven, the stopper inserted, secured by a steel spring, and connected with the ʻU-tube and burette ; the slightly greased stopcock H_a is inserted and the air is displaced from the vessel and burette. Whilst the vessel cools in a current of nitrogen for five to seven minutes, the substance is weighed out.

The cooled vessel is then removed ; the pipettes are allowed to cool whilst nitrogen is passed through, and the substance is brought from the balance into limb *B*. Either 1 c.c. of pyridine or 3 c.c. of anisole are added to dissolve it ; if it dissolves completely,[1] 0·5 c.c. of Grignard reagent are pipetted into limb *A*, the upper half of the stopper is well greased with vaseline, the steel spring hooked in, and the reaction vessel closed as before. The vessel is then suspended in the water which has been removed from its container, and the mercury is adjusted between the marks 0·0 and 0·1 of the burette. Whilst nitrogen is passed through the apparatus for five minutes, the weighing tube is weighed ; the pipettes are cleaned, for the next determination, with methylated spirit and acetone [2] and placed in the drying-oven. The stopcocks H_a and H_b are now closed and the current of nitrogen stopped. If a pressure change is perceptible after three minutes, atmospheric pressure is set up again by opening

[1] One attempts to dissolve undissolved material by warming carefully in an atmosphere of nitrogen.

[2] The pipettes for the Grignard solution are first cleaned with water acidified with hydrochloric acid and with water.

the cock H_b. After three minutes more no further alteration in pressure should be established.[1]

The height of the mercury, the barometer reading and the temperature of the water are noted, and the Grignard reaction carried out as follows :—

The water-bath is first removed and the Grignard solution • allowed to flow on to the substance by tilting the reaction vessel through about 60–70 degrees ; the vessel is shaken round till no more evolution of gas can be ascertained in the burette.[2] Then the excess pressure produced by the evolution of methane is removed by lowering the bulb of mercury, and the reaction vessel is again placed in the water-bath. After five minutes the volume is read accurately to 0·005 c.c., with the three mercury levels at the same height. If the reaction is carried out at higher temperatures, the reaction vessel is placed in a suitable water-bath and frequently shaken. After five minutes the water-bath is exchanged for one at room temperature ; the temperature is checked and read after ten minutes.

The vessel is taken off, the vaseline removed by benzene, and the apparatus cleaned as before. The reaction vessel and pipettes are dry again in fifteen minutes. The first analysis must always be preceded by a test analysis or blank determination. If no more analyses are to be made, the burette must be protected against moisture with a small tube of phosphorus pentoxide. The purity of new reagents must be checked by a blank test.

Calculation

Taking 0·7168 gm. as the weight of 1 litre of methane at N.T.P., the percentage of active hydrogen is given by the equation :—

$$\text{percentage of hydrogen} = \frac{1 \cdot 0078 \times 100 \times 1000 \, v_0}{22365 \cdot s} \qquad (1)$$

$$= 4 \cdot 506 \times \frac{v^0}{s}$$

$$\log (\text{factor}) = 0 \cdot 65381.$$

$$v_0 = \text{reduced volume in c.c.}$$

$$s = \text{weight of substance in mgm.}$$

If the factor f_N of the nitrogen tables is used for the reduction to 0° C. and 760 mm. p, the calculation of v_0 may be omitted. Accordingly we have :—

$$\text{percentage of hydrogen} = 3 \cdot 604 \, f_N \cdot \frac{v}{s}.$$

$$\log (\text{factor}) = 0 \cdot 55673.$$

[1] If a decrease in volume is observed, this may be corrected by leading nitrogen through again, the excess pressure due to moisture cannot usually be corrected.

[2] The reaction is usually complete in one minute.

If the work has been carried out in pyridine at higher temperatures, the value obtained by blank determination must be considered, as well as the vapour pressure of the pyridine. For the calculation of f_N, the vapour pressure of pyridine at the corresponding temperature, given in the following table, must be subtracted from the barometric reading :—

VAPOUR PRESSURE OF PYRIDINE [1]

t °C.	p (mm.)	t° C.	p (mm.)	t° C.	p (mm.)
10	7·7	18	13·4	26	20·8
12	9·0	20	14·9	28	23·2
14	10·2	22	16·7	30	25·7
16	11·8	24	18·6	32	29·0

Examples :

Glucose	t	v_0	Percentage of active hydrogen found.
3·145 mgm.	21° C.	1·93 c.c.	2·77
1·520 mgm.	21° C.	0·93 c.c.	2·76

Glucose, $C_6H_{12}O_6$, contains five active hydrogen atoms per molecule.
Molecular weight = 180·08.
Active hydrogen. Theory. 2·78 per cent.

Sulphosalicylic acid.	t	v_0	Percentage of active hydrogen found.
2·175 mgm.	22° C.	1·32 c.c.	2·73
2·545 mgm.	21° C.	1·56 c.c.	2·76

Crystallised sulphosalicylic acid, $C_7H_6O_6S + 2H_2O$, contains seven active hydrogen atoms.
Molecular weight = 254·12.
Active hydrogen. Theory. 2·75 per cent.

IV. Determination of Primary Amino Groups, by van Slyke's Method [2]

Basis of the Method

The method depends on the fact that many primary amino groups react with nitrous acid according to the equation :—

$$RNH_2 + HNO_2 = ROH + H_2O + N_2.$$
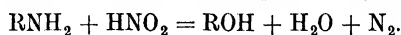
[1] Taken from : *Jahrestabellen chemischer, physikalischer, biologischer und technologischer Konstanten und Zahlenwerte*, Vol. 9, p. 179. 1929.
[2] *J. of Biol. Chem.*, **9**, 195 (1911); **12**, 275 (1912); **16**, 121 (1913); **23**, 407 (1915), and " Handbuch der biologischen Arbeitsmethoden," Division I., Part 7, p. 263.

The nitrous acid is prepared from sodium nitrite and glacial acetic acid. The oxides of nitrogen thus liberated are used to drive all air out of the apparatus, before adding the material to be analysed. The oxides of nitrogen are absorbed in a Hempel pipette by means of alkaline permanganate solution and the remaining nitrogen is volumetrically determined in a measuring burette.

It reacts most readily [1] with all α-amino acids and the peptides derived from them in less than five minutes at room temperature ; in lysine the reaction of the second amino group, in the ε-position, is only complete in thirty minutes. Because the nitrogen of the pyrrolidine, indole, and imidazole ring does not react, only half of the total nitrogen of tryptophane, of histidine, only one-third, and of arginine, one-fourth, is available. No nitrogen is split off from proline and oxyproline. The guanidine group NH_2 . $C(NH)NH$— does not react at all in free guanidine nor in creatine and arginine. Methylamine and ammonia require shaking for one and a half to two hours. With urea the reaction is only complete in eight hours. Amino-purine and amino-pyrimidine must be shaken for two to five hours. With aspartic acid, only one amino group reacts ; the amido nitrogen remains unattacked. Glycocoll and cystine yield a volume of gas which is rather too high ; that from glycocoll is usually 103 per cent., and that from cystine 107 per cent., of the theoretical amount. Cephaline,[2] unhydrolysed, gives 110 per cent. of the theoretical amount.

Reagents

Sodium Nitrite Solution. Thirty grams of sodium nitrite (A.R.) are dissolved in 100 c.c. of water. Small amounts of nitrogen are liberated from even the purest commercial preparations on acidifying, which must be determined as on p. 170.

Glacial Acetic Acid. A.R.

Alkaline Permanganate Solution. Fifty grams of potassium permanganate and 25 gm. of caustic potash are dissolved in 1 litre of water.

Secondary Octyl Alcohol, from Kahlbaum, Berlin.

Apparatus

The van Slyke micro-apparatus shown in Fig. 50 is made by the firm of " Halle Laboratory Apparatus G.m.b.H." By means of particularly large stopcocks, the apparatus is made completely air-tight. It consists of three principal parts—a reaction vessel with

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[1] *Cf.* E. Abderhalden : " Handbuch der biologischen Arbeitsmethoden," Division I., Part 7, p. 263 ; and G. Klein : " Handbuch der Pflanzenanalyse," H. Lieb, I., p. 234. (Berlin : Julius Springer, 1931.)

[2] H. Rudy and L. H. Page : *Z. physiol. Chem.*, **193**, 251 (1930).

burette and funnel for filling it, a measuring burette, and a Hempel pipette.

The reaction vessel D is cylindrical and has a capacity of about 6 c.c. At the bottom it is constricted and possesses an attached tube with glass cock d, for running off the solution. Before the reaction vessel passes into the capillary, of 1–1·5 mm. bore, above it,

a bulb is formed which prevents the liquid from being shaken into the capillary. After about 8 cm., the capillary is bent at right angles and leads to a three-way cock c. This makes the connection to the measuring burette F, on the one hand, and, on the other, its lower tube serves for drawing off the gases from the reaction vessel or the measuring burette. A capillary bent downwards, sealed to the cock c, is connected, by rubber tubing, with the Greiner-Friedrich cock f of the measuring burette.

Close above the lower constriction of the reaction vessel, on the right, there opens the capillary three-way cock b, which connects upwards with the burette B, and possesses a short delivery tube which bends downwards. The capacity of the burette is 2 c.c. and it is subdivided into 0·01 c.c. The 14 c.c. cylindrical funnel A, provided with stopcock a, opens out of the left side of the reaction vessel at the same height.

FIG. 50.—van Slyke micro-apparatus, by the firm of Halle Laboratory Apparatus, G.m.b.H., Halle a. S.

D, Reaction vessel. B, Burette. A, Funnel. F, measuring burette. G, Hempel pipette.

The reaction vessel and funnel each possess a filed mark. That of the reaction vessel marks about two-fifths of the total volume and that of the funnel is so placed that the volume up to it is one-fifth of the volume of the reaction vessel.

The cock d of the reaction vessel is connected with a four-way piece K, by means of rubber tubing. The horizontal pieces of the four-way piece are connected with the cocks b and c by means of rubber tubing. A long piece of tubing, open at the bottom, is drawn over the lower piece of K.

The measuring burette has a capacity of 3 c.c., is about 30 cm.
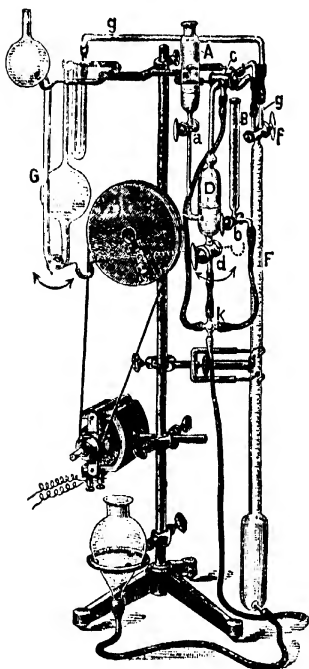
high, possesses an inner diameter of 3–5 mm., and is subdivided in 0·01 c.c. Below the cock f it is narrowed to a capillary 2–3 cm. long, in which the zero is placed. At the end of the graduation it widens into a reservoir for the absorption of the gases of the reaction, especially the oxides of nitrogen. Just above the bottom it is connected, by rubber tubing, with the levelling bulb. The Greiner-Friedrich cock f is connected with the bridge g by rubber tubing over the upper piece. The bridge consists of a thick-walled horizontal capillary, bent downwards at both ends, and forms the connection between the burette F and the Hempel pipette G. The Hempel pipette consists of two glass bulbs, at different heights, each 70 mm. in diameter; the lower one is connected with the bridge by a capillary bent twice through 180 degrees.

The whole apparatus is suspended on a stand. Only the burette, motor, and belt of the pulley are fixed tightly. The reaction vessel hangs only on two forks at the right and left of c. The Hempel pipette is also fixed at the same height.

The whole apparatus must be so made that the stopcock d and the lower bend of the Hempel pipette are at the same level; the pulley of the motor which controls the shaking is fixed between these parts of the apparatus. The guiding-rod may be placed radially on the pulley, so that the amplitude, and therefore the shaking, may be carefully regulated. The speed of shaker may be further regulated by the resistance of the motor.

Weighing

The substance is weighed into a 2 c.c. or 5 c.c. graduated flask by means of the weighing tube for nitrogen (p. 79), dissolved in water, and made up to the mark. The strength of the solution is such that 1 or 2 c.c. will yield 0·5–2·0 c.c. of nitrogen. It must be remembered that, in this method, an amino group yields twice as much nitrogen as in the Dumas method. If the substance is insoluble in water a solution in 2 N mineral acid, 50 per cent. acetic acid, or dilute alkali may be used.

Course of the Analysis

The apparatus, which has been dried after cleaning with chromic-sulphuric acid, distilled water and alcohol is mounted on the stand; the rubber connections are made with very tight-fitting tubing and the cocks greased well with high-vacuum fat. The Hempel pipette is then filled with potassium permanganate solution up to the rubber connection, distilled water is brought into the measuring burette from the levelling bulb, and both are connected by means of the bridge g, which is full of water; the whole should be free from air.

By lowering the bulb, one tests whether air-bubbles are formed by the connection ; in any case, the permanganate solution is driven through g up to f, the air from the burette is driven out up to c, and water is again brought into the bridge by raising the levelling bulb. The reaction vessel and the capillary of the burette are then connected through f, and the reaction vessel is filled with water from the measuring burette up to the cock c, which is then closed' against the burette. Before beginning the displacement of the air, the cock b under the filling burette B must be closed. The bore must be empty.

For driving out the air with oxides of nitrogen, glacial acetic acid is poured into the funnel A up to the mark and allowed to flow into the reaction vessel D. In the same way, as much sodium nitrite solution is added as will fill the reaction vessel up above the bulb ; the air can escape through c towards the four-way piece K. The cock c is then closed against the reaction vessel, which is shaken with a open until the oxides of nitrogen push the mixture in it down to the mark. The oxides of nitrogen are allowed to escape through c and the shaking is repeated to remove the last traces of air. After the gas has been drawn off, the solution is again displaced to the mark, a is closed, and the connection with F is made through c whilst the levelling bulb is in the low position.

The solution of the substance is now transferred from the graduated flask to the burette B with an ungraduated pipette and adjusted exactly on the zero by running it out through b. An exact amount of the solution is now drawn carefully into the reaction vessel by the reduced pressure due to the low position of the levelling bulb, and shaking is begun. The time required for shaking depends on the nature of the substance analysed ; usually three to five minutes are necessary. With very strongly frothing substances 0·5–1 c.c. of octyl alcohol are brought into the reaction vessel from the burette B on removal of the air, or it is added in the same way immediately froth appears in the analysis. The burette must first be well rinsed out with glacial acetic acid.

After the shaking is finished, the cock a is opened and the mixed nitrogen and oxides of nitrogen are driven into F. The cock f is turned through 180 degrees, and by raising the levelling bulb the whole of the gas is driven from the burette into the Hempel pipette. The absorption by permanganate of the oxides of nitrogen and of the carbon dioxide liberated is accelerated by slowly shaking the Hempel tube for two minutes.

The residual gas, which consists of pure nitrogen, is again driven into the measuring burette, adjusted at the zero, and the volume read as usual to 0·002 c.c., after levelling to atmospheric pressure.

The nitrogen is then run off through c into the atmosphere and the whole determination carried out again from the beginning of the shaking. If the reaction has been quantitative, the volume read the second time is no greater than that in the blank determination.

The blank test is made immediately before or after the corresponding analysis, under exactly the same conditions but without the material analysed, because the volume of nitrogen obtained in the blank test depends not only on the purity of the reagents, but also, within certain limits, on the time of shaking. With good solutions of nitrite, 0·03 c.c., or at most 0·05 c.c., of nitrogen are produced on shaking for five minutes. With the same reagents the blank test is constant as a rule, and it is sufficient to check it before every new series of analyses.

Calculations

The difference between the volumes of nitrogen found in the determination and in the blank test is divided by two, and the temperature and barometric pressure are read.

The nitrogen is calculated as in the Dumas method, using the table for the reduction of gas in Küster's logarithmic tables.[1] Because water is here used as the sealing liquid, the vapour pressure of water at the temperature of the reading must be subtracted from the barometric pressure.

$$\log \text{(percentage of nitrogen as } NH_3)$$
$$= \log v + \log N + 2 - \log \text{(mgm. of substance)}$$

Example :

From 2·748 mgm. of alanine,[2] at 21° C. and 757 mm. pressure, 0·798 c.c. of nitrogen were obtained. After subtracting the blank value of 0·04 c.c. and dividing by two, $v = 397$ c.c. The partial pressure of water vapour at 21° C. is 18·7 mm., approx. 19 mm. ; $\log N$, at 738 mm. $= 0·05210$.

Molecular weight of alanine, $C_3H_7NO_2 = 89·06$.

$$\therefore \text{ Nitrogen} \begin{cases} \text{Theory,} & = 15·73 \text{ per cent.} \\ \text{Found,} & = 15·55 \text{ per cent.} \end{cases}$$

V. Determination of Methoxyl and Ethoxyl Groups, by F. Pregl's Method

Gravimetrically, by the Micro-Zeisel Method

The Zeisel method depends on the splitting off of methoxyl or ethoxyl compounds with boiling hydriodic acid, with formation of

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[1] F. W. Küster : " Logarithmische Rechentafeln," Editions 35 to 40, p. 60, Table 7, 1929.

[2] 5·496 gm. of alanine were dissolved in 2 l.c., and 1·00 c.c. was drawn from the filling burette.

the alkyl iodides. The volatile alkyl iodides pass, in carbon dioxide, into a receiver containing alcoholic silver nitrate solution. They react with this to form a double salt, $AgI.AgNO_3$. By the addition of water and nitric acid, the double salt is split up into silver nitrate and silver iodide and weighed as the latter.
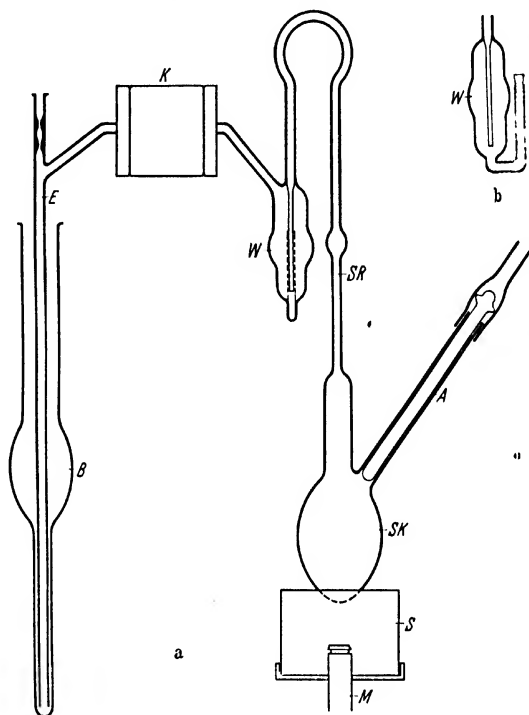
Fig. 51.—*a*, Apparatus for the micro-determination of methoxyl. *b*, Washer with filling tube. (⅓ actual size, *SK* ⅔ actual size.)

M. Micro-burner with chimney, *S*, away from flame. *SK*. Boiling flask with ascension tube *SR*, and side tube *A* with small sealed tube and rubber tubing. *W*. Washer. *E*. Delivery tube. *B*. Absorption vessel. *K*. Cork for holding tube in the clamp.

Apparatus

This consists of apparatus for the evolution of carbon dioxide and of the apparatus for the analysis (Fig. 51), with the absorption vessel *B*.

The carbon dioxide is obtained from the usual Kipp generator or from steel bottles of carbon dioxide ; it is freed from hydrochloric acid vapour by passing through sodium carbonate solution in a wash-bottle. A few pieces of string are inserted, to a distance of 10 cm., into the tubing, of 4–6 mm. bore, which connects the

waste bottle with the apparatus for the determination, and a screw pinchcock is provided at this spot; it is thus possible to adjust the gas current with extreme accuracy.

The apparatus for the determination is made of Jena glass. It consists of the olive-shaped flask *SK*, of 4–5 c.c. capacity, with the ascension tube *SR* and the side tube *A* through which the material and the carbon dioxide are introduced. In order to prevent escape of vapours into *A* during the determination, its volume is reduced by the insertion of a loosely fitting small glass tube, sealed at both ends. To prevent this from slipping into the flask, it is furnished, as in the diagram, with a small collar through which the carbon dioxide can pass. If the side tube terminates at a somewhat oblique angle, any obstruction to the gas current is avoided.

The ascension tube *SR* of the boiling flask widens, about 4 cm. above the flask, to a small bulb, which acts with the tube as an air condenser. About 6–8 cm. above the bulb the tube is bent over as in the illustration and is sealed into the washer *W*. It is advisable not to fill the washer from below as before, but to add the washing liquid through the filling tube, as shown in Fig. 51, *b*, into the apparatus which is already clamped.

The washer is connected with the gas inlet tube *E* through a small tube passing obliquely upwards, then horizontal, and finally slanting downwards. The tube *E* is open at both ends. Around the horizontal part of the small tube a split perforated cork is bound with copper wire, to enable the apparatus to be held in a clamp. The tube *E* has two constrictions above the point where it joins the connecting tube. Before using the apparatus, a small drop of water is placed in the upper opening of *E*. The capillary constrictions prevent this from running down. The tube is then closed at the top with a small cork. With this water seal it is possible, in the simplest way, to make a joint which is impermeable to the vapours of alkyl iodides. At the end of the distillation of the alkyl iodide the delivery tube is rinsed down after removal of the cork.

As receiver, a test-tube *B*, which is widened in the middle, is used. The narrow lower part has a diameter of 7–8 mm., and a length of 50 mm. In the proportionally narrow space between this and the delivery tube the bubbles only rise slowly and the alkyl iodide is quantitatively absorbed in the alcoholic silver nitrate solution.

Into every new absorption vessel 2 c.c. of water are brought and the meniscus is marked. For the analyses, the alcoholic silver nitrate solution is filled exactly to this mark.

Reagents

Hydriodic Acid, of sp. gr. 1·7, which has been most carefully prepared for the determination of methoxyl by the Zeisel method so that it can be used directly, can now be obtained from the firms of E. Merck, of Darmstadt, and Kahlbaum, of Berlin. All conditions which promote the decomposition of hydriodic acid and separation of iodine, such as exposure to light, and more particularly access cf air, should be carefully avoided. Often, after standing for a long time, preparations which have become deep brown and opaque yielded too low values for methoxyl, because the concentration of the hydriodic acid was lower than that corresponding to sp. gr. 1·7,[1] owing to its decomposition. In order to have fresh hydriodic acid always at hand, only small consignments of 25 or 50 gm. should be ordered.

In very exceptional cases it might be necessary to use hydriodic acid of sp. gr. 1·96, as, *e.g.*, for the determination of glycerol in aqueous solutions, in order to bring these up to the specific gravity of the mixture of constant boiling-point through the addition of strong hydriodic acid. According to some published statements, if Pregl's instructions for phenol-acetic anhydride are to be retained, satisfactory results can only be obtained with hydriodic acid of sp. gr. 1·96. In spite of these statements a warning against the use of this must be given, because hydriodic acid is distilled from the flask till the concentration of the constant boiling mixture is attained, and the solution of thiosulphate in the washer is insufficient for the quantitative absorption of this.

Alcoholic Silver Nitrate Solution. Four grams of silver nitrate (A.R.) are dissolved in 100 gm. of 95 per cent. alcohol in a 200 c.c. round-bottomed flask and the solution boiled for four hours on a water-bath under a reflux condenser. After standing for two days the liquid is decanted from the partly separated silver into a brown stock-bottle.

A. Friedrich [2] made the observation that the alkyl iodide was quantitatively transformed into silver iodide by freshly prepared alcoholic silver nitrate solution, but that after a few days too little silver iodide was obtained. Because the silver nitrate solution is constant after about a week, and after this time the error remains practically constant, according to Friedrich for 2 c.c. of alcoholic silver nitrate solution 0·12 mg. must be added. With this empirical correction, for six to ten months excellent results are obtained. After this time, as Friedrich had already ascertained, the correction

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[1] Stoll, Hofmann, and Helfenstein : *Helvet. chim. acta,* **17,** 641, note p. 654 ; *cf.* A. Friedrich : *Mikrochem.,* **7,** 191 (1929).

[2] *Z. physiol. Chem.,* **163,** 141 (1927) ; *cf.* also : "Die Praxis der quantitativen organischen Mikroanalyse," p. 141.

no longer suffices—*e.g.*, vanillin gives a result which is too low by 1–2 per cent.[1]

It is therefore not advisable to prepare too large a stock of alcoholic silver nitrate solution, but only as much as will probably be required for six to eight months. From about a week to at least six months the correction of 0·06 mgm. of AgI per cubic centimetre qf the silver nitrate solution is quite reliable.

Phenol (A.R.).

Acetic Anhydride.

Nitric Acid (sp. gr. 1·4) free from halogens (p. 99).

Tinfoil. The thickness should be such that a sheet of the size of one pfennig weighs no more than 12 mgm.

Cadmium Sulphate Solution, 5 per cent.

Sodium Thiosulphate Solution, 5 per cent.

Course of the Analysis

Before each analysis the apparatus must be carefully cleaned and dried. This is done by attaching the connection from the pump to the side tube and drawing about 200 c.c. of tap water, followed by 100 c.c. of distilled water, through the apparatus, keeping the finger on the filling tube of the washer. After wiping the outside, the apparatus is dried in the oven at 120° C. Drying may be considerably accelerated by drawing acetone through the apparatus after the distilled water. The delivery tube must be perfectly clean and fat-free, in order that the precipitate may be removed quantitatively. For this purpose the dried apparatus is clamped in a cork in the stand and the delivery tube immersed in warm sulphuric-chromic acid for at least five minutes. Meanwhile the material is weighed out.

Because it is essential that the substance should be completely dissolved, the solubility of unknown substances is tested. Two or three crystals of the material and about one-third of the amount of phenol and acetic anhydride required for the analysis (p. 177) are placed in a test-tube. The test-tube is placed in a water-bath at 50°–60° C. If the substance dissolves completely, the determination may be carried out without further preliminaries. If the material remains undissolved, the test-tube is heated to boiling over the small flame of a micro-burner. If the substance then goes into solution, the weighed-out material in the boiling flask must similarly first be dissolved in boiling phenol and acetic anhydride and the hydriodic acid added only after cooling. If the material is wholly or partially insoluble in the amount of solvent corresponding to that usually

[1] According to A. Friedrich, the same phenomena had also been observed at the Institute of Prof. H. Fischer in Munich.

used for the analyses, its solubility in more phenol (two to three times that on the tip of the spatula) and more acetic anhydride (1 or 2 drops) is tested. It is helpful to powder the substance very finely in an agate mortar and then to place it in a small cup of tinfoil, without compressing this.

According to the preceding instructions, it has always been possible, so far, to bring even substances which are very difficult to dissolve into reaction with hydriodic acid in homogeneous solution.

Solids, 3–5 mgm., are weighed into a cup of tinfoil. This is made from a square piece of tinfoil, of about 16 mm. edge, which is cut round with the scissors or rounded with a spatula over a coin. It is then put over the end of a glass rod, 5 mm. in diameter, with rounded edges, and pressed against this, and the rod pressed on the notebook used for the analysis. The cup thus formed is removed by turning the rod and is then weighed. After weighing, it is placed on the notebook, the substance to be analysed is added, brushing off any residue which adheres to the outside, and the cup placed on the balance to ascertain if the weight is approximately correct. Finally, it is compressed to a tetrahedron with three fingers of the right hand, weighed after one minute, and then carried to the apparatus on a copper block in the desiccator. If the preliminary test has shown that the material should first be dissolved in the boiling flask, the cup is very slightly compressed, so that it may fall into the dried flask through the side tube which is held vertically. If some materials stick to the walls they are rinsed down with a little acetic anhydride.

Liquids. For weighing liquids which do not volatilise undecomposed into the cooler part of the apparatus, the micro-weighing bottle is used (Fig. 35). While a glass thread is used for transferring oils, liquids are sucked high into a fine capillary, a drop is allowed to fall into the micro-weighing bottle, and the stopper inserted. A small cup of tinfoil, about 12 mgm., must still be used for transferring the material to the flask. Hydriodic acid, which is otherwise inclined to bump, boils perfectly quietly under the influence of the separated stannous iodide, and addition of fragments of porcelain, etc., is superfluous. It is, however, important to see that the tinfoil never weighs more than 20 mgm., because too large amounts of tin would reduce the concentration of the hydriodic acid so much that too low methoxyl values would be obtained. This obviously applies also to the tinfoil cups for solids.

The still empty apparatus is removed from the clamp and the delivery tube rinsed down inside and out, first with distilled water and then with alcohol. For this a drop of distilled water is introduced into the upper opening with a wash-bottle or glass rod and the cork

then inserted immediately in order to form the water seal already mentioned. The apparatus is then clamped loosely so that the boiling flask hangs about 20 mm. above the micro-burner. The washer is charged through the filling-tube with about 0·5 c.c. of 5 per cent. cadmium sulphate and 0·5 c.c. of 5 per cent. sodium thiosulphate solutions, and the tube closed with a small cork.

• After the receiver has been washed with water, distilled water, and alcohol, it is filled up to the 2 c.c. mark with alcoholic silver nitrate solution and the delivery tube is introduced by turning the apparatus outwards about the tube placed horizontally in the cork. The receiver is so placed, if necessary by placing a support underneath it, that the delivery tube ends about 1 or 2 mm. above the bottom. The ascending bubbles are flattened or even burst and rise slowly in the solution, and the completeness of the absorption of the alkyl iodide is ensured.

The boiling-flask is charged through the side-tube. The weighed material, in tinfoil, is transferred by a platinum-tipped forceps, to the oblique arm of the flask and allowed to slide down it. To the solvent are then added a few crystals of phenol, twice on a spatula, 4 or 5 drops of acetic anhydride from a dropping pipette and finally 2 c.c. of hydriodic acid.

If the preliminary test has shown that the substance must first be dissolved, the flask is carefully warmed to boiling with a very small flame of a micro-burner, before adding the hydriodic acid. To prevent superheating, the ascending tube is tapped with a pencil or wooden rod ; bumping at the beginning of the boiling is thus avoided.

As soon as the material is completely dissolved, the solution is cooled well and the acid added. Immediately afterwards the small sealed tube is pushed with rubber tubing into the mouth of the side-tube. With the screw pinchcock closed, the cock of the Kipp apparatus is next opened and the speed of the bubbles is so adjusted by the pinchcock that in the alcoholic silver nitrate solution there are never more than two bubbles rising at the same time.

A very small non-luminous flame of a micro-burner with chimney is now placed at a distance of about 15 mm. below the boiling flask. The consequent warming produces an acceleration of the gas current, and it would be a mistake to alter the pinchcock accordingly, as after boiling commences the ascending bubbles resume their original rate. After about three minutes the first indications of formation of a precipitate at the lower end of the delivery tube are observed. The precipitate, which is at first coarsely flocculent, gradually becomes crystalline, and does not usually show any apparent increase after eight or ten minutes. In spite of this, it is better to allow the

liquid to boil for about twenty minutes, so that the last traces of methyl iodide may pass over into the receiver.

The operation is concluded by removing the burner, raising the apparatus with the clamp so that the end of the delivery tube only reaches up to about one-third into the wider portion of the receiver, removing the cork from the water seal, and rinsing the delivery tube from above, inside and out, with water acidified with nitric acid and with alcohol. Should a few particles of silver iodide still adhere to the delivery tube, this is again rinsed alternately with these reagents. Should a particle of any size be obstinately retained at any point it can be removed with the feather (p. 118). The contents of the receiver are diluted by the washings so that their final level is at about the middle of the widened portion. After adding 5 drops of concentrated nitric acid free from halogen, the receiver is placed in a gently boiling water-bath until the contents begin to boil and the silver iodide which has collected into a ball sinks to the bottom ; this requires at least two minutes.' The use of more nitric acid is to be avoided, as it may lead to too high results. These may also occur, however, if washing the delivery tube with water acidified with nitric acid is omitted. In pure alcoholic solution, especially, the silver halide double compound, AgI. AgNO$_3$, is not quantitatively decomposed on the water-bath in the usual time of one or two minutes.

After cooling under the tap, the separated silver iodide is aspirated into a filter-tube in the manner already described (p. 103) and weighed.

Calculation

1 mgm. of silver iodide corresponds to 0·1321 mgm. of OCH$_3$.

1 mgm.   „   „   „   0·1918 mgm. of OC$_2$H$_5$.

log (percentage of OCH$_3$ or OC$_2$H$_5$) = log (weight of AgI)
   + log (factor) + 2 − log (weight of substance).

$$\log 0·1321 = \bar{1}·12096.$$
$$\log 0·1918 = \bar{1}·28287.$$

Examples :

Vanillin (C$_8$H$_8$O$_3$) :

   3·750 mgm. : 5·78 mgm. AgI = 20·37 per cent. OCH$_3$.
          Theory   = 20·40 per cent. OCH$_3$.

The method fails with substances containing sulphur, because the cadmium sulphate solution of the washer does not retain sulphuric acid quantitatively. Similarly, even with a 10 per cent. cadmium sulphate solution, alkyl groups attached to sulphur cannot be determined. Besides methoxyl and ethoxyl groups, other

lower alkyl groups bound to oxygen also yield silver iodide. Glycerol readily forms isopropyl iodide and can thus be quantitatively determined.

The method is also excellent for the detection and determination of alcohol of crystallisation.

The Volumetric Determination by H. Lieb,[1] as recommended by A. Kirpal and Th. Bühn.[2]

As described on p. 175, the determination is carried out in F. Pregl's methoxyl apparatus (p. 172), only the alkyl iodide formed is absorbed in pyridine in two receivers connected with one another. The compound formed with pyridine is dissolved in water after evaporating off the excess of pyridine ; a minimal amount of potassium chromate is then added and the iodine titrated with N/100 silver nitrate solution.

The Volumetric Determination by the Method of F. Vieböck and C. Brecher [3]

Basis

The method depends on the collection of the alkyl iodide formed with hydriodic acid, in the receiver in a solution of sodium acetate in acetic acid, to which a few drops of bromine have been added. The following reactions occur :—

$$CH_3I + Br_2 \rightarrow CH_3Br + IBr.$$
$$IBr + 3H_2O + 2Br_2 \rightarrow HIO_3 + 5HBr.$$
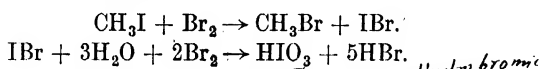
The sodium acetate present neutralises the hydrochloric acid formed and the excess of bromine is decomposed by formic acid. Finally, the iodate formed is titrated, as in Leipert's [4] determination of iodine (p. 115), with N/30 or N/50 sodium thiosulphate solution after the addition of potassium iodide.

Reagents

Hydriodic Acid.[5] Sp. gr. = 1·70.
Phenol. A.R.
Sodium Thiosulphate Solution. 5 per cent.
Sodium Acetate in Glacial Acetic Acid. 10 per cent.
Bromine (free from iodine).
Formic Acid. 80–100 per cent.
Aqueous Sodium Acetate Solution. 20 per cent.

[1] 2nd (English) edition of this book, p. 188.
[2] *Monatshefte f. Chemie,* **36**, 853 (1915).
[3] *Ber. dtsch. chem. Ges.,* **63**, 3207 (1930).
[4] *Mikrochem.,* Pregl-Festschrift 266 (1929).
[5] See p. 174.

Potassium Iodide Solution.   10 per cent.
Sodium Thiosulphate Solution.   N/50. Prepared as on p. 114.
Starch Solution.   Prepared as on p. 209.

Course of the Analysis

The analysis is carried out in Pregl's apparatus for the determination of methoxyl. The delivery tube of the apparatus is not washed with alcohol, but only with distilled water (better, distilled twice), the water seal is formed and the washer charged with the 5 per cent. sodium thiosulphate solution. In the receiver which has been finally washed with distilled water, 2 c.c. of the 10 per cent. sodium acetate solution are placed, and 4 or 5 drops of bromine are added. In order to prevent the bromine vapour from entering the laboratory, a piece of cotton-wool slightly moistened with formic acid is placed at the mouth of the receiver. The filling and heating of the boiling flask is conducted exactly as for the gravimetric determination.

After boiling the hydriodic acid for thirty minutes, the reaction is complete. The delivery tube is rinsed well, inside and out, with distilled water, and the inside of the receiver is rinsed quantitatively into a 100 c.c. Erlenmeyer flask, with ground-in stopper, into which 5 c.c. of the 20 per cent. sodium acetate solution have previously been placed ; 2 drops of formic acid are then allowed to flow along the wall, the flask is shaken around and formic acid added until the solution is colourless. After the addition of 4–6 drops of formic acid there is usually no bromine to be detected even by the smell. (On testing with methyl red, see p. 115.)

Now, 2 c.c. of the 10 per cent. potassium iodide solution are added, the solution is acidified with 5 c.c. of 2 N sulphuric acid and the liberated iodine is titrated after two minutes, as described on p. 115.

One cubic centimetre of N/50 sodium thiosulphate corresponds to 0·6204 mgm. of OCH_3 or to 0·9008 mgm. of OC_2H_5.

Calculation

log (percentage of alkoxyl) = log (c.c. N/50 sodium thiosulphate) + log (factor) + 2 − log (mgm. of substance).

Factor for OCH_3 = 0·10341 ; log factor = $\bar{1}$·01458.
Factor for OC_2H_5 = 0·15013 ; log factor = $\bar{1}$·17647.

For multiple determinations, the method is quicker than the gravimetric, and with reagents which, in the blank test, consume practically no sodium thiosulphate (*i.e.*, only 0·02 c.c.) it gives accurate values. With vanillin, the results are about 0·1–0·2 per

cent. below the theoretical. If substances are to be tested for traces of alcohol of crystallisation, the gravimetric method is preferable, because the smallest amounts of silver iodide can be determined by it and the carrying out of the analysis depends on these.

Formerly, considerable fluctuations were observed at the Kaiser-Wilhelm-Institut by the volumetric method. The analytical results were too low if only 2 drops of bromine were present, while acetic anhydride caused results to be too high. Since 4 or 5 drops of bromine have been added and the addition of acetic anhydride has been given up, results have been satisfactory.

In order that no hydriodic acid may be consumed unnecessarily, many authors advise placing the material in the boiling-flask in small glass cups and adding red phosphorus to prevent bumping. If the substance is weighed in cups of tinfoil and one wants to carry out two analyses with one filling of the boiling flask, then, according to A. Friedrich, another 0·5 c.c. of hydriodic acid must be added after introducing the second weight. With natural products of unknown composition, as well as with substances possessing alkoxyl groups which are difficult to decompose, it is necessary to split off the alkyl group with a considerable excess of hydriodic acid. It is, therefore, advisable to use fresh hydriodic for each determination.

Determination of Methoxyl and Ethoxyl Groups in the Presence of one another

The methods of F. Pregl and F. Vieböck and C. Brecher which have been described give us no information as to whether the alkyl iodide formed has been obtained from a methoxyl or an ethoxyl group. To decide which is present, or if both are present simultaneously, several methods are at our disposal.

Separation of Methoxyl and Ethoxyl Groups, by the Method of W. Küster and W. Maag [1]

Basis of the Method

Although this process requires from 8 to 20 mgm. of material, it is drawn up in the first place for substances of which the constitution is still completely unknown. Then it makes it possible to separate the alkyl iodides quantitatively split off according to Zeisel's principle and to weigh them.

The method is based on the process of R. Willstätter and M. Utzinger.[2] The alkyl iodide formed is collected in an alcoholic

[1] *Z. physiol. Chem.*, **127**, 190 (1923).
[2] *Ann.*, **382**, 148 (1911).

solution of trimethylamine ammonium iodide ; by this, tetramethyl ammonium iodide or trimethyl-ethyl ammonium iodide are formed. Because the former is almost insoluble in absolute alcohol and the latter is readily soluble, the salts may readily be separated. After the addition of dilute nitric acid and silver nitrate the iodine is precipitated as silver iodide and weighed.

Apparatus

In order that the alkyl iodide may be completely absorbed in the alcoholic solution of trimethylamine, F. Pregl's apparatus must undergo some modification. To obtain the smallest possible bubbles of gas, the inlet capillary of the washer is constricted to 0·5 mm. The apparatus has two receivers. The first, which is connected with the washer by a ground-in joint, consists of a small test-tube of 11 mm. diameter and 65 mm. high. It contains a spiral with inlet and delivery tubes on the principle of an extraction apparatus. The spiral has five coils with two depressions, one above and one below, in order to obstruct the bubbles for some time. The second receiver is connected with the first by a ground-in joint. It consists of a test-tube, of 3 mm. bore and 90 mm. high, in which the inlet tube is so ground that the escaping bubbles are flattened. Both inlet tubes for gas should be of 0·5 mm. bore at their ends.

Course of the Analysis

The material to be analysed is weighed into a cup of tinfoil (p. 176) and placed in the boiling-flask, where phenol, acetic anhydride, and hydriodic acid are added as previously described.[1] The first receiver is then charged with 3 c.c. of alcoholic trimethyl amine solution, which is prepared from 5 c.c. of a 10 per cent. solution of trimethylamine and 12 c.c. of absolute alcohol. In the second receiver, 1 c.c. of this solution is then placed. Now the apparatus is connected with the Kipp apparatus and slowly filled with carbon dioxide from this. The delivery of carbon dioxide is then held up with a pinchcock and the boiling-flask slowly heated to 140° C. in a bath of sulphuric acid so that one, or at most two bubbles per second rise in the receiver. This rate of bubbling is maintained throughout the analysis. If no more bubbles rise in the receiver after warning, the pinchcock is carefully opened and carbon dioxide passed through the apparatus at the rate of one bubble per second.

After half an hour the flame is turned out, the receiver removed and kept stoppered for a day in order to enable the tetramethyl ammonium iodide to crystallise. For the separation of the alkyl

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[1] If the substance is difficult to dissolve, it must be dissolved before addition of hydriodic acid. *Cf.* p. 175.

ammonium iodide salt the spiral is first removed from the receiver, placed in a small beaker, and rinsed with absolute alcohol. After the delivery tube has been similarly rinsed, the contents of both receivers are transferred to a beaker by repeated rinsing with alcohol ; the beaker is finally evaporated to dryness on a water-bath and allowed to cool in a desiccator. The trimethyl-ethyl ammonium .iodide is dissolved from the crust of salt with 3–4 c.c. of absolute alcohol and filtered through a filter moistened with this. The last traces of the iodide are dissolved by washing the precipitate three times more with absolute alcohol. To the alcoholic filtrate are added two to three times its volume of water, 2–4 drops of nitric acid free from halogen, 3 c.c. of 1 per cent. silver nitrate solution, and the silver iodide is precipitated on the boiling water-bath, collected in the small filter-tube (p. 103), dried, and weighed.

For the determination of the methyl group, the tetramethyl ammonium iodide is dissolved from the filter with hot water, the spiral and inlet tube are rinsed, and all the washings are collected in the beaker used for the evaporation. The precipitation and weighing of the silver iodide follows as above. The method is accurate to $\pm$ 1 per cent. of theory.

According to research by A. Friedrich, good results are obtained as long as the weight of substance taken is not too small, i.e., not less than about 5 mgm.

At the Kaiser-Wilhelm-Institut the usual apparatus for methoxyl or methylimino groups has been used successfully for the detection of the alkyl, using the double receiver (cf. second English edition of this book, p. 188). The precipitate was collected in a small centrifuge tube and identified by determination of carbon and hydrogen.

Methoxyl and ethoxyl groups may also very often be quantitatively distinguished in a simple manner by the method of R. Kuhn and H. Roth,[1] described on p. 201, for the determination of methyl groups attached to carbon.

If the substance analysed by the micro-Zeisel method is oxidised with chromic acid, no acetic acid is found when only methoxyl is present. In the presence of an ethoxyl group, an amount of acetic acid equivalent to the silver iodide weighed is formed. If, e.g., the substance contains one group of each alkoxyl per molecule, one molecule of acetic acid is obtained from the ethoxyl group only. This method is modified if other methyl groups attached to carbon, acetyl, or benzoyl groups are present.[2]

A further determination of methoxyl and ethoxyl groups when

[1] *Ber. dtsch. chem. Ges.*, **66**, 1274 (1933).
[2] On further application, see p. 201.

both are present has been published by A. Friedrich.[1] It is carried out on the following principle :—

First, the oxygen of the alkoxyl groups is calculated from the iodine determined by Pregl's method, or, as iodate, by the method of Vieböck and Brecher.

On a second weight, the alkyl iodide formed in the same way is not led into the receiver, but directly into a micro-combustion tube, for the determination of carbon and hydrogen in which the alkyl iodide is burnt to carbon dioxide and water. The tube contains red-hot platinum contacts. The carbon dioxide is absorbed by soda-lime as in the carbon determination. From the increase in weight of the soda-lime the carbon content of the alkoxyl groups may be calculated. From these results the proportion of oxygen atoms to carbon atoms in the alkoxyl groups may be calculated. If the ratio $O : C = 1 : 1$, a methoxyl group is present, if $1 : 2$, an ethoxyl group, and if $2 : 3$, one methoxyl group to one ethoxyl group.

VI. Determination of Methyl and Ethyl Groups attached to Nitrogen. Methods of F. Pregl and H. Lieb

History, Theory, and Experiments

After the methoxyl method had been worked out, in 1913 F. Pregl had begun the investigation of the determination of methyl groups attached to nitrogen. For this the principle of the Herzig and Meyer [2] macro-method was retained. By means of hydriodic acid, the alkyl imino substance was converted into the quaternary ammonium salt, which on heating to 300°–360° C. was decomposed with separation of the alkyl iodide.

Because the decomposition does not as a rule take place exactly according to schedule, it is necessary to repeat the experiment two or three times. In 1915 Pregl and Lieb continued the research, and the apparatus shown in Fig. 52 was devised as suitable for the purpose.

Since then, this process, which is important for the organic chemist, has been very thoroughly studied by various professional colleagues. S. Edlbacher,[3] A. Friedrich,[4] F. Vieböck and C. Brecher,[5] K. H. Slotta and G. Haberland [6] may here be specially named. The process, which is not very easily handled, might be improved by small alterations and the certainty of the determination considerably

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[1] *Mikrochem.*, **7**, 185 (1929).
[2] *Ber. dtsch. chem. Ges.*, **27**, 319 (1894); *Monatshefte f. Chemie*, **15**, 613 (1894); **16**, 599 (1895); **18**, 379 (1897).
[3] *Z. physiol. Chem.*, **101**, 278 (1918).
[4] *Mikrochem.*, **7**, 195 (1929).
[5] *Ber. dtsch. chem. Ges.*, **63**, 3207 (1930).
[6] *Ber. dtsch. chem. Ges.*, **65**, 127 (1932).

increased ; above all, by the use of Friedrich's apparatus (Fig. 53), in which the flow of gas is not hampered by the condensed hydriodic acid.

As S. Edlbacher discovered, the washer originally charged with a suspension of red phosphorus does not completely absorb the carbon dioxide and the vapours of hydriodic acid and hydrogen .sulphide which pass through it ; precipitation of silver sulphate in the receiver may easily occur, particularly in the case of sudden increase in the speed of the gas. For this reason Edlbacher has used two washers instead of one, and filled both with a 5 per cent. solution of cadmium sulphate. This suffices to retain completely both hydriodic acid and the hydrogen sulphide which occurs in the commercial hydriodic acid.

If a mixture of 5 per cent. cadmium sulphate and 5 per cent. sodium thiosulphate, as proposed by Friedrich, is placed in the washer, the alkyl imino determination may be carried out gravimetrically in both types of apparatus illustrated.

Through the volumetric process of F. Viebӧck and C. Brecher, this method has undergone very considerable simplification, because injuries from hydrogen sulphide do not occur and therefore commercial hydriodic acid for the Zeisel methoxyl determination can be used as long as its concentration is not lowered to less than 1·70. It therefore saves the placing of cadmium sulphate in the receiver. It is advisable to keep the thiosulphate solution. Slotta and Haberland use a 5 per cent. solution of sodium thiosulphate to which a little sodium carbonate is added. Methyl groups attached to sulphur may also be very accurately determined volumetrically by the methods described later. Investigations which were started to determine these groups gravimetrically failed even if 10–15 per cent. cadmium sulphate solution was used as washing liquid and carbon dioxide passed very slowly, at the rate of a bubble in one and a half seconds, because precipitation of silver sulphide always occurred.

In decomposition products of lactoflavine (vitamin B_2) and also with synthetic flavines,[1] much too low and fluctuating results were obtained with the prescribed methods for determination of methylimino groups. If these substances were previously dissolved to clear solutions by proportionately large amounts of phenol and acetic anhydride by warming or even by rather slow boiling, and the ammonium iodide, gold chloride, and hydriodic acid only added after cooling, the analyses are excellent. The view widespread in the literature on this, that methylimino groups which are difficult to split off owing to their particular linkages are the source of too low

[1] R. Huhn and H. Roth : *Ber. dtsch. chem. Ges.*, **67**, 1458 (1934).

results, is therefore open to question. Further investigations permit of the quantitative determination of such groups after the substance has been previously dissolved, *e.g.*, ξ-trimethyl-pentadecabetaine.[1]

Owing to this knowledge, we propose to dissolve every substance [2] before the analysis, because this precaution, which only requires five to seven minutes, may prevent disappointment. The number of distillations required is not reduced by this process, nor can this. reduction be established by the addition of gold chloride.

F. Pregl, and also later communications from professional colleagues, have drawn attention to the fact that previous dissolution of the substance is, in many cases, a condition of the quantitative course of the reaction. Because it is the task of this book to describe a method of work obtained experimentally which permits us to analyse any substance whatever, the dissolution of the substance before the addition of the hydriodic acid in the course of the work is prescribed.

In connection with work in the Kaiser-Wilhelm-Institut which is still unfinished, the necessity arose of checking oxazoles and thiazoles free from methyl groups attached to nitrogen, as well as several of their derivatives, by a normal determination of methyl-imino groups by the method of Vieböck and Brecher. From these investigations, which are still unfinished, the interesting observation should be communicated that the consumption of thiosulphate by thiazole picrate corresponds to exactly one CH_3 group per molecule, and with 2-aminothiazole to one CH_3 group for four molecules of substance. With dimethylthiazole, α, μ-dimethyl- and β, μ-dimethyl-thiazole, no thiosulphate was used up.

The Apparatus of F. Pregl and H. Lieb

This is made of Jena glass and consists of the small flask *SK*, of about 20 mm. diameter, and the somewhat slanting distilling tube *SR*, which is 150–160 mm. long and of 6–7 mm. outer diameter. According to Pregl, thicker distilling tubes make repeated distillations necessary, whilst with the given dimensions even with the first distillation the result is almost quantitative. The small tube, *A* of Fig. 52, is at least 100–140 mm. long, in contrast with that of the methoxyl apparatus. The sealed tube is inserted as in the methoxyl apparatus. The distilling tube *SR* bends round, proceeds slightly downwards for 60 mm., and then descends vertically into the receiver *V*. The almost horizontal portion, 60 mm., is surrounded by a split cork, as in the methoxyl determination, so

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[1] R. Kuhn and F. Giral : *Ber. dtsch. chem. Ges.*, **68**, 387 (1935).

[2] With the obvious exception of routine analyses of substances which are readily soluble.

that the whole apparatus may be supported by a clamp at this point.

The receiver V for the distilled hydriodic acid consists of two bulbs, which are connected with one another, in order completely to avoid spirting of the hydriodic acid. The washer W, which follows, differs from the washer of the methoxyl apparatus, being surmounted by two wider portions, which are so large that on sucking back the hydriodic acid before beginning a fresh distillation the whole contents of the washer are retained by them.

The gas delivery tube E which follows the washer is exactly the same as in the apparatus for the determination of methoxyl, and the same form of widened test-tube B is used for holding the alcoholic silver nitrate solution.

The apparatus is heated in a stamped copper or iron pan K, of 50 mm. diameter and 50 mm. in height, which is filled with powdered copper oxide residues from the old combustion tubes. The temperature can be accurately read on a thermometer Th dipping into the copper oxide.

Fig. 52.—Apparatus for the determination of methylimide. (Pregl and Lieb method.)

K, Copper or iron bath filled with fine copper oxide, the temperature of which is measured by the thermometer Th. SK, Boiling-flask. SR, Condenser tube. V, Receiver. W, Washer. E, Delivery tube. B, Widened test-tube.

The Jena glass apparatus permits fifty to eighty analyses to be carried out with one flask.

A. Friedrich's Apparatus

(Fig. 53.)

In contrast with the apparatus of Pregl and Lieb, the stream of gas no longer passes through the condensed hydriodic acid. Therefore, no hot vapours enter the washer, and the rate of bubbling which has once been set up remains the same throughout the determination. If a temperature of 360° C. has been reached, one may even leave the apparatus to itself.

At the beginning of the determination the gas current can pass

along either *AA'* or the way from *B*, the cock controlling *BB'* being open. Later, when hydriodic acid has condensed and collected in *A*, the passage through *B* is alone available. Apart from its normal bore, the cock possesses a groove which goes vertically downwards and ends before the middle of the bore. If the cock is turned clockwise through 90 degrees from the position shown in diagram, the passage of gas is cut off and at the same time *B'* is • placed in communication with the outside air.

Like the preceding apparatus, the whole is made of Jena glass. Two such pieces of apparatus [1] have been in operation for some
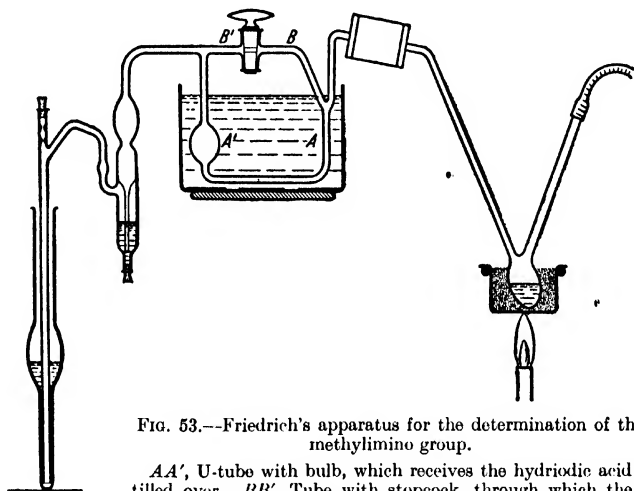
FIG. 53.—Friedrich's apparatus for the determination of the methylimino group.

AA', U-tube with bulb, which receives the hydriodic acid distilled over. *BB'*, Tube with stopcock, through which the gas current passes into the water.

years in the Kaiser-Wilhelm-Institut. Until now only one flask has cracked, so that there is no occasion for using a quartz flask instead of a glass one.

Reagents

The required reagents are as stated on p. 174 for the gravimetric determination of methoxyl, moreover there are : *Ammonium iodide,* and a 5 *per cent. aqueous solution of gold chloride* which is added with a dropping pipette.

Course of the Gravimetric Determination of Alkylimino Groups
In the Apparatus of Pregl and Lieb

The apparatus is cleaned exactly as in the methoxyl determination and similarly provided with the water seal. With the long-

[1] Made by the firm of P. Haack, Vienna.

handled weighing tube (p. 79), the material to be analysed is placed in the boiling-flask through the tube *A*, which is held horizontally, and the apparatus is then clamped in the cork at a height such that the copper or iron pan can be heated with the very small flame of a micro-burner. The washer is then filled about one-third full of equal volumes of sodium thiosulphate and the
• cadmium sulphate solution and closed. Three to five spatula points of phenol and 3–5 drops of acetic anhydride are added through the side-tube and the substance dissolved as on p. 175 by carefully warming with a micro-burner. If the amount of solvent added should still be insufficient, the addition of phenol especially may be increased up to double the amount.

After the flask has cooled again, the delivery tube is dipped into the receiver, which holds exactly 2 c.c. of alcoholic silver nitrate solution. To the dissolved substance there are added two to three spatula points of ammonium iodide (*i.e.*, about ten to twenty times as much as the amount of material weighed), a small ball of tinfoil, 1 or 2 drops of gold chloride—the last is not absolutely essential—and finally 2 c.c. of hydriodic acid. With the pinchcock closed, the tubing of the Kipp apparatus is drawn over the side-tube ; the tubing contains the small sealing tube. The gas current is regulated as in the methoxyl determination so that not more than two bubbles rise in the receiver at the same time. The boiling-flask is heated in the copper or iron pan, which contains the thermometer.

If the heating is regulated so that the hydriodic acid is only just at the boil, about 140° C., it is possible to determine the methoxyl or ethoxyl groups only, in substances containing alkoxyl groups.

The path, however, which the alkyl iodide has to travel to the receiver is longer than in the methoxyl determination. The exchange of the receiver for a fresh should not be made until after forty to forty-five minutes. During this manipulation the heating should not be interrupted. The heating which follows is best made according to S. Edlbacher's directions. First the temperature is quickly raised to 160° C. with the Bunsen flame. The further heating up to 200° C. must be very carefully undertaken for twenty to twenty-five minutes, because in this range of temperature the hydriodic acid distils from the boiling-flask into the receiver. If the heating is too rapid, vapours of hydrogen sulphide and hydriodic acid enter the washer with the carbon dioxide, are not completely retained in it, and the analysis is spoilt. Finally, the bath of copper oxide is heated with a larger flame to 350°–360° C. and kept at this temperature for at least half an hour. The experiment is completed by extinguish-

ing the flame and continuously maintaining a stream of carbon dioxide whilst the gas inlet tube is well rinsed into the lowered receiver with water acidified with nitric acid and with alcohol. The precipitate is treated as in the methoxyl determination and the receiver is kept, protected from halogen vapour, in a test-tube stand.

Before beginning to suck back the hydriodic acid for the second distillation, it is most advisable to empty the washer and add fresh solutions. If the temperature of the copper oxide bath is below 100° C., the hydriodic acid is carefully sucked back into the flask, in such a manner that no washing liquid is drawn back into the receiver.

For this purpose a rubber tube is slipped over the side tube of the boiling-flask and the hydriodic acid is slowly sucked back into the flask. The determination is then repeated. The distillations are to be repeated until the separation of the silver iodide double compound which appears is only just perceptible, and the quantity formed is not more than 0·5 per cent.

A. Friedrich's Apparatus

Because this apparatus has been used most satisfactorily for years in the Kaiser-Wilhelm-Institut, " its manipulation shall be described in so far as it differs from the apparatus described above.

Until the beginning of the heating the determination is prepared exactly as with the apparatus of Pregl and Lieb. The cock is so adjusted that BB' is open. As soon as heating begins, the U-shaped piece of the apparatus is immersed in a glass dish (Fig. 53), which must be full of hot water throughout the determination in order to prevent condensation in this part of the apparatus.

After the determination, the stream of carbon dioxide is stopped with the pinchcock and the glass cock turned so that communication is established between B' and the outer air ; the contents of the flask are cooled and the distilled hydriodic acid thus aspirated back into the flask, without any danger of also aspirating back the contents of the receiver or the washer. If the boiling-flask should already be too cold, the hydriodic acid is sucked back by means of tubing. After suction, a few drops of hydriodic acid still remain in AA'. If one wishes to bring even this residue into the flask, the delivery tube is rinsed out, while some hydriodic acid is allowed to flow in from a pipette through the groove and then slowly aspirated through as before. Renewal of the washing liquid is unnecessary.

Volumetric Determination of Alkylimino and Alkyl Groups attached to Sulphur

Method of F. Vieböck and C. Brecher [1]

For this the apparatus shown in Figs. 52 and 53 are used. The boiling-flask is charged as for the gravimetric determination. As in the volumetric determination of methoxyl, 5 per cent. sodium thiosulphate is placed in the washer, to which 0·5 per cent. sodium carbonate is added as suggested by K. H. Slotta and G. Haberland.[2] The receiver is charged as on p. 189, and the iodate is titrated after each distillation as on p. 115. When only 1 or 2 drops of thiosulphate are required for the titration the analysis is regarded as completed.

Recently, determinations of alkylimino and thiomethyl groups have been carried out both with the absorption solutions of Vieböck and Becher and of Slotta and Haberland. In both cases the results are very good, so that the volumetric method is now preferred to the gravimetric, especially as the latter sometimes fails with methyl groups attached to sulphur. For the volumetric determination of alkylimino groups, N/50 sodium thiosulphate solution is always used.

<div style="text-align:center">Calculation</div>

(a) *Gravimetric*

log (percentage of alkyl) = log (weight of AgI)
$$+ \log (\text{factor}) + 2 - \log (\text{weight of substance}).$$
For CH_3, Factor = 0·06398 ; log Factor = $\bar{2}$·80604.
For C_2H_5, Factor = 0·12380 ; log Factor = $\bar{1}$·09273.
Atropine, $C_{17}H_{23}NO_3$; mol. wt. 289·18, 5·740 mgm.
1st distillation, 4·16 mgm. AgI
2nd distillation, 0·54 mgm. AgI
$$\overline{4·70 \text{ mgm. AgI}} = 5·24 \text{ per cent. } CH_3$$
Theory for   1 CH_3 = 5·20 per cent. CH_3.

(b) *Volumetric*

log (percentage of alkyl) = log (No. of c.c. of N/50-$Na_2S_2O_3$ used) + log (factor) + 2 − log (mgm. of substance).
For CH_3, Factor = 0·3005 ; log Factor = $\bar{1}$·47780.
For C_2H_5, Factor = 0·5808 ; log Factor = $\bar{1}$·76403.

VII. The Micro-determination of the Acetyl and Benzoyl Groups

Method of R. Kuhn and H. Roth [3]

While at Innsbruck in 1910–1913, F. Pregl began to develop the quantitative determination of acetyl groups in small amounts of

[1] *Ber. dtsch. chem. Ges.*, **63**, 3207 (1930).
[2] *Ber. dtsch. chem. Ges.*, **65**, 127 (1932).
[3] *Ber. dtsch. chem. Ges.*, **66**, J274 (1933).

substance. His first attempts were carried out in conjunction with H. Lieb, according to the principle of Wenzel's process. In 1924, with M. Baltadschiewa, he proposed to drive over the acetic acid in an atmosphere of hydrogen under diminished pressure into N/100 alkali. Sulphur dioxide and other volatile acids affect the results.

F. Kögl and J. J. Postowsky[1] saponify the substance with barium hydroxide, acidify with phosphoric acid, and distil the acetic acid into the receiver in an atmosphere of water vapour. With some experience the limit of error was easily kept down to ± 0·5 per cent.

K. Freudenberg and E. Weber[2] have recommended alcoholic *p*-toluene sulphonic acid for the determination of the acetyl group in sugars and catechin derivatives. The acetic acid thus formed is esterified by the excess of alcohol present and distilled off as ethyl acetate. This operation is often repeated after the addition of fresh alcohol. The ethyl acetate is collected in caustic soda and saponified ; the alcohol is distilled off, and the acetic acid determined. This simple method, which has not yet been tested for acetyl groups attached to nitrogen, gives excellent results in the hands of specialists About 20 mgm. of substance is required. With substances containing halogens the silver salt of *p*-toluene sulphonic acid is added. ·

F. Pregl and A. Soltys[3] used the above *p*-toluene sulphonic acid, in a 25 per cent. solution in water, as saponifying agent, distilling the ethyl acetate, however, through a silver condenser *in vacuo* (12 mm. Hg.) into N/100 sodium hydroxide. The small amounts of sulphur dioxide formed during saponification were retained by primary sodium phosphate and an accuracy of ± 0·5 per cent. was obtained for weights of 3–6 mgm. of the substance analysed.

According to A. Friedrich and S. Rapoport,[4] the iodimetric determination of acetic acid is preferable to the alkalimetric.

A. J. Bailey and R. J. Robinson[5] have considered the distilling off of the ethyl acetate to be unnecessary and have saponified the substance in a Pyrex flask with dilute sodium hydroxide. After saponification the excess of alkali is determined and from the difference the acetyl content of the substance is calculated. Apart from the long time required for saponification (twelve to thirty-five hours), the claim for this process as an acetyl method fails, because the authors have disregarded all the difficulties of earlier authors which afterwards led to the separation of the acetyl from harmful products of saponification ; lactones, lactames, and non-volatile

[1] *Ann.*, **440**, 34 (1924).
[2] *Z. angew. Chem.*, **38**, No. 13, p. 280.
[3] Second (English) edition of this book, p. 107 ; also, *Mikrochem.*, **7**, 1 (1929).
[4] A. Friedrich and S. Rapoport, in joint investigations with O. Watzlaweck, found that after the driving over of the water, owing to the vacuum, the very fine dry primary sodium phosphate is frequently carried over into the alkali present; *Biochem.*, **251**, 432 (1932).
[5] *Mikrochem.*, **15**, 233 (1934).

products of decomposition are thus determined as acetyl by this process.

All the above processes, except the last-named, have made very accurate determinations possible for experts. They are, however, not easy to manipulate, especially if such analyses are only carried out occasionally, by organic chemists or by students of organic microanalysis. The following method, by F. Kuhn and R. Roth,[1] has made it possible to determine acetyl and benzoyl groups by acid, alkaline, and alcoholic saponification within the required degree of accuracy of $\pm$ 0·3–0·5 per cent. in a simple manner. The apparatus was first used for the determination of methyl groups attached to carbon by oxidation with chromic acid. After the substance has been saponified in a small Jena glass flask, the acetic or benzoic acid is distilled off at atmospheric pressure from solution in sulphuric acid, through a quartz condenser, and titrated.

F. Pregl [2] writes : " According to investigations with A. Soltys, in all distillations through glass and quartz condensers, incorrect results were necessarily obtained." According to a personal communication of Dr. A. Soltys, in his time investigations were made with a quartz condenser which was not completely transparent. The quartz condenser used for the last three years in the Kaiser-Wilhelm-Institut at Heidelberg has been found absolutely satisfactory. According to F. Emich,[3] in the conditions of the acetyl determinations, no traces of silicic acid could be detected in 200 c.c. of distillate either with malachite green or with methylene blue. In consequence of the observation by F. Pregl and A. Soltys, the quartz condenser was tested in the above manner for errors which were likely to occur in the analysis.

In order to avoid the formation of sulphur dioxide which had already been observed by F. Pregl, it was natural to use phosphoric acid instead of sulphuric acid for the saponification and distillation. Unfortunately all experiments with this were a failure, because phosphoric acid was always to be found in the distillate. In 100 c.c. of distillate—i.e., ten distillates of 10 c.c. each—according to H. Lieb 1·6 mgm. of phosphoric acid could be determined. The acetic acid could therefore not be driven off from primary sodium phosphate solution. If the acetic acid was distilled from solutions containing sulphuric acid, more or less sulphur dioxide was always to be found in the distillate. It was satisfactory to find iodimetrically that the sulphur dioxide was completely expelled from the distillate after boiling for three or four seconds. A. Friedrich and S. Rapoport[4]

[1] *Ber. dtsch. chem. Ges.*, **66**, 1274 (1933).
[2] *Mikrochem. Z.*, **7**, 1 (1929).
[3] *Mikrochemisches Praktikum*, 2nd Edition, p. 98, 1932.
[4] *Biochem. Z.*, **251**, 432 (1932).

had already in exhaustive researches on the removal of carbon dioxide from solutions of acetic acid without loss of acetic acid, found seven to eight seconds to be an optimal time of boiling ; this time is amply sufficient for the removal of all sulphur dioxide. The boiling up necessary before the titration ensures not only the expulsion of carbon dioxide but also of sulphur dioxide.

Apparatus

The bubble-counter is the usual one with sealed-on U-tube which is used for the determination of carbon and hydrogen. Charged with 50 per cent. sodium hydroxide, it serves for the
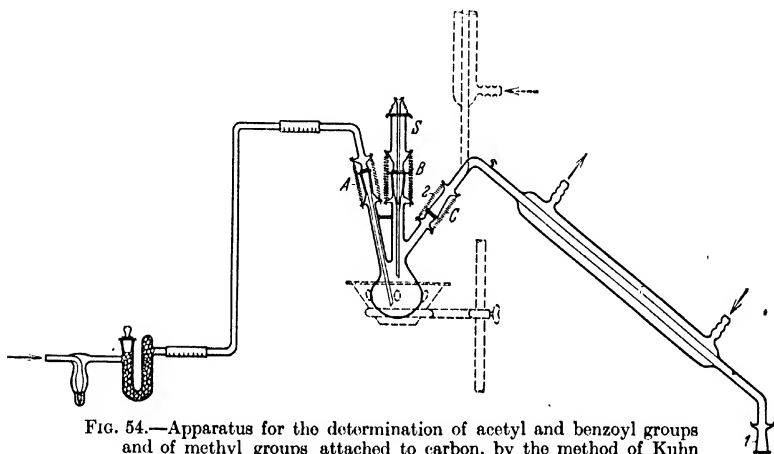
FIG. 54.—Apparatus for the determination of acetyl and benzoyl groups and of methyl groups attached to carbon, by the method of Kuhn and Roth.

A, *B*, *C*, limb of the saponification and oxidation flask. For the heating under reflux condenser, the ground joint *1* of the quartz condenser is connected with *C*; if the joint *2* is connected, the condenser acts as a descending condenser, *S*.

purification of the oxygen, and more particularly for measuring the speed of the gas. The U-tube is filled with soda-lime or ascarite.

The Jena glass flask is pear-shaped, of 45 c.c. capacity, and possesses three limbs, *A*, *B* and *C*, with ground-in joints of the types shown in diagram. Through the limb *A*, 80 mm. long and 6 mm. bore, there passes the gas inlet tube, of 2 mm. bore, which is widened to fit the joint at the top. The tube reaches almost to the bottom of the flask. The bubbles escaping from it prevent super-heating. The limb *B* is 80 mm. long, the mantel-joint of 10 mm. diameter holds the stopper with funnel inside it. The funnel reaches slightly below the bottom of the limb. With the glass rod *S* inserted it has a capacity of 8 c.c. ; marks are placed at 2 and 7 c.c. By raising the ground-in glass rod, water may be added without interrupting the distillation. A steel spring or a rubber band

presses the glass rod back again afterwards so that no more water can flow in. To keep the apparatus firm, the limbs A and B are connected by a sealed-on glass rod. The limb C branches from the same place as A and B; it is 65 mm. long and 50 mm. bore. It is inclined to the horizontal at 50 degrees; its ground-in end forms an absolutely air-tight joint with the quartz condenser. All the ·stoppers are secured with steel springs. The substance is saponified by immersing the flask in a boiling water-bath, consisting of a litre beaker, so that the water reaches up to the bottom of the limbs; for the oxidation with chromic acid it is better to heat in a small Babo funnel.

The quartz condenser is made from completely clear, transparent quartz and has ground-in joints at both ends, which exactly fit into the limb C. The total length is 36 cm.; 4·5 cm. from the joint 1 the condenser is bent through 40 degrees. If the joint 1 is connected with C, the condenser acts as a reflux condenser. At a distance of 5·5 cm. from the joint 2 the condenser is bent through 90 degrees in the same direction as for 1. If 2 is inserted in C, a descending condenser is formed.

· The apparatus is made by the firm of W. Velter, Heidelberg.

Reagents [1]

Sodium Hydroxide Solutions. 5 N and N.

Sodium Hydroxide in Methyl Alcohol. N. Four grams of sodium hydroxide, in tablet form, are dissolved in 50 c.c. of water + 50 c.c. of methyl alcohol. To remove rather small amounts of acid, methyl alcohol is usually first refluxed for about fifteen minutes with solid potassium hydroxide and then distilled off.

Wenzel's Sulphuric Acid. One hundred cubic centimetres of sulphuric acid, sp. gr. 1·84, are added to 200 c.c. of water.

p-Toluene Sulphonic Acid, as made for scientific work by E. Merck, Darmstadt. Twenty-five per cent. solution in water.

* *5N Chromic Acid.* One hundred and sixty-eight grams of Merck's anhydride (for the determination of carbon) is dissolved in 1 litre of water and filtered through a finely pored fritted glass suction filter.

* *Sulphuric Acid,* sp. gr. 1·84.

* *Phosphoric Acid,* sp. gr. 1·7, of the consistency of syrup.

* *Hydrazine.* Ten cubic centimetres of hydrazine hydrate are mixed with 10 c.c. of water.

Metaphosphoric Acid. Prepared from phosphorus pentoxide and a few drops of water.

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[1] Reagents marked * are only necessary if acetyl groups are to be determined as " methyl groups attached to carbon " according to the method described in the chapter which follows (p. 201).

Barium Chloride. Crystallised.

N/100. *Sodium Hydroxide Solution.*

N/100. *Hydrochloric Acid.* Kept in microburettes with automatic zero adjustment.

Phenolphthalein. One per cent. solution.

Choice of Type of Saponification

It is decisive for the success of the analysis that the solubility of the substance to be analysed and the strength of the linkage of the acetyl or benzoyl groups shall be taken into account. Particular attention must be paid to the possibility that, besides acetic or benzoic acid, other volatile acid decomposition products may be formed.

The solubility of a very small sample of the substance is first examined in a test-tube in the four given saponification agents. If the acyl group is combined with oxygen it is usually saponified on the boiling water-bath in twenty minutes with sulphuric acid or *p*-toluene sulphonic acid, or in fifteen minutes with sodium hydroxide in water or methyl alcohol. There are, however, compounds in which the group is attached to oxygen which must be saponified with sodium hydroxide in methyl alcohol for two and a half hours, *e.g.*, the methyl ester of triacetyl-cholic acid, acetylated catechin, antho-cyanidine, etc., yield too high values with the alkaline saponification ; acetyl-salicylic acid gives exactly twice the theoretical result, because, besides acetic acid, the salicylic acid is also titrated.[1] In this and similar cases if other methyl groups attached to carbon are absent, it is essential that the chromic acid method described in the following chapter should be chosen ; by this, the volatile acids which occur in addition to acetic acid are destroyed by oxidation.

Compounds, in which acetyl or benzoyl groups are attached to nitrogen, are usually saponified with alkali in methyl alcohol, to avoid an unnecessarily long time of saponification.

It is only very rarely that substances occur which are insoluble in the reagents at our disposal for the saponification. In such cases the substance is first dissolved in 1 c.c. of pure pyridine (p. 162), sodium hydroxide in methyl alcohol added as before and the pyridine and methyl alcohol are distilled off after the saponification which follows. The pyridine which still remains in the saponification flask is fixed as sulphate after acidifying and does not affect the determination.

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[1] Substances which undergo auto-oxidation by which volatile acid decomposition products are formed with water vapour, are saponified and distilled in a current of nitrogen for preference.

Course of the Analysis

If the apparatus is new or has not been used for some time, it is first treated with warm sulphuric-chromic acid to remove any impurities present.

All the glass parts are then cleaned, before every determination, .with distilled water, and dried by warming and evacuating with the water-pump or in the drying-oven at 110° C. The condenser is cleaned similarly, but not dried.

The Weighing

It has been found best in practice to weigh out as much substance as will require 3–6 c.c. of N/100 acid for the titration. From the long-handled weighing tube (p. 79), the substance is transferred to the flask.

Substances which are difficult to dissolve are previously powdered as finely as possible in the agate mortar. Substances which previously were quantitatively dried, are allowed to slide in a boat to the bottom through limb *B* by slanting this. Syrupy liquids are treated similarly. Liquids are weighed by J. Pirsch's method (see p. • 242). The capillary is placed point downwards in the saponification agent, which is already in the flask, or in the sulphuric-chromic acid solution. The condenser must have been previously put on. The capillary is broken by pressing on the handle with a glass rod. If the rod is moistened by this it is rinsed with 1 c.c. of water. The ground-in funnel is quickly inserted and secured.

Assembling the Apparatus and the Saponification

The bubble-counter, which is fixed as usual to a micro-stand, is connected with an oxygen or nitrogen gasholder by rubber tubing. The velocity of the gas current is adjusted by a needle-valve or a pinchcock to fifty bubbles per minute. The saponification agent chosen is allowed to flow on to the substance in the flask from the corresponding pipette, *i.e.* :—

For acid saponification, 1 c.c. of Wenzel's sulphuric acid or 1 c.c. of the *p*-toluene sulphonic acid solution ; for alkaline saponification, 1 c.c. of 5 N sodium hydroxide or 4 c.c. of N sodium hydroxide in methyl-alcoholic solution.

The flask is firmly clamped by the limb *B*, the joint of *C* moistened with water, the condenser fitted tightly as a reflux condenser and secured with the steel springs. The condenser is clamped in the middle. The joint of the gas inlet tube and the outer joint of the small funnel are now moistened with metaphosphoric acid, inserted, and each is held by two steel springs. •Afterwards the connection

with the bubble-counter is made, the glass rod S is tightly fitted and the funnel charged with 1–2 c.c. of water from the wash-bottle.

The substance is now saponified in a prepared water-bath—a litre beaker. The water is kept boiling by means of a burner ; it should reach the neck of the flask.

Distillation and Titration

The factor of the N/100 sodium hydroxide is determined in conditions which approximate as closely as possible to those of the titration to be made later. Into the 100 c.c. quartz flask, as used by Pregl for the Kjeldahl determination (p. 91), 3–4 mgm. of pure oxalic acid ($+ 2H_2O$) were placed.[1] This is dissolved in 20 c.c. of distilled water, the carbon dioxide is boiled off in seven seconds while shaking round over an open flame, and 4–5 drops of the 1 per cent. solution of phenolphthalein are added. This is titrated rapidly with N/100 alkali till a pink colour is just formed and the reading taken. The next drop (0·01–0·02 c.c.) should produce a definite coloration.

If the saponification is finished, the water-bath is removed and allowed to cool ; the glass rod S is taken out ; the condenser is rinsed into the flask with 4–6 c.c. of water from a wash-bottle, and the glass rod is replaced. The condenser is then removed and washed carefully with 100–200 c.c. of water. It is then replaced as a descending condenser with all connections absolutely tight, and secured with the springs. If the saponification has been carried out with alkali in methyl alcohol, 5 c.c. alcohol are distilled off to remove methyl alcohol and the condenser rinsed as before and replaced. The clamp at B should be loosened.

In the funnel, to neutralise the acid : (a) 1 c.c. of 5 N sodium hydroxide is added from a pipette after the saponification with sulphuric acid ; (b) 0·5 c.c. of N sodium hydroxide is added after saponification with p-toluene sulphonic acid. After hydrolysis by alkali, 1 c.c. of Wenzel's sulphuric acid is placed in the funnel. By carefully raising the glass rod the alkali or acid is allowed to flow into the flask and 2–3 c.c. of water are used to rinse down the funnel. Finally, for the first distillation, water is added up to the mark 7 in the funnel, and from two to four small pieces of pumice are placed in the flask. The limb B is then clamped tightly, the bubble-counter is connected, and the funnel is fitted in the joint, after smearing with metaphosphoric acid. The following distillation of the acetic or benzoic acid holds good for the determination of acetyl or benzoyl groups and also for the determination of acetic

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[1] The weight is so chosen that, as in the actual experiment, 3–6 c.c. of N/100 alkali are used for the titration.

acid obtained by oxidation. The distillates are collected in a 25 c.c. Jena glass measuring cylinder with a funnel. For driving over the acids, it is best to warm in a small Babo funnel, with a flame adjusted so that 5–6 c.c. of distillate condense in five minutes throughout the whole distillation. When the volume in the flask has been concentrated to 2–3 c.c., water is slowly run into the ·flask, to mark 2, by raising the glass rod S ; the distillation is not interrupted. This addition is similarly repeated until the end of the determination. After the first distillate, consisting of four portions of 5 c.c., has been collected, the measuring cylinder and funnel are removed immediately after a drop has fallen and the quartz flask is quickly slipped under the end of the condenser. The funnel is then placed on this flask and the distillate is poured into the flask from the measuring cylinder. The measuring cylinder is replaced under the end of the condenser, without rinsing, and the flask is taken away for the titration. Whilst the distillation is taken further, two or three crystals of barium chloride are placed in the quartz flask, which is boiled as in the determination of the factor, observing whether the solution remains clear, and titrated after the addition of 4 or 5 drops of phenolphthalein. The further distillates are similarly tested in the absence of sulphuric acid. If turbidity, due to barium sulphate, is observed after boiling, which only occurs very rarely through heavy superheating, the analysis must be considered spoilt.

If less than 4 c.c. of N/100-NaOH are used up in the titration of the first 20 c.c., then the next 10 c.c. of distillate are titrated. If the amount of acetic acid found is larger, 15 c.c. of distillate are collected for the titration. If the volume in the distillation flask is concentrated to 2–3 c.c. before the distillation of each 5 c.c. portion, then after the first two titrations, as may be seen from the example given later, approximately 1 per cent. of the acetic acid still remains behind. When the end-point appears with the first drop (0·01–0·02 c.c.) of standard alkali, the analysis is finished. The whole distillation requires thirty to forty minutes.

The acetic or benzoic acid may be determined in one titration on all the distillates together, but this is not advisable. Even the first attempts at obtaining the acetic or benzoic acid quantitatively at atmospheric pressure in the smallest possible distillate, besides the systematic investigations at driving over these acids without traces of sulphuric acid, led to the specified method of fractional titration, which has been retained for the following reasons also :—

1. If no acetic or benzoyl groups can be detected, the analysis is finished with the first titration and all further distillations are spared.

2. From the titration of the first 20 c.c., the number of further distillations still necessary may be estimated.

3. If, in consequence of superheating, sulphate is detected in the distillate from the third or fourth distillation, almost the whole of the acetic or benzoic acid has already been titrated in the previous satisfactory distillates and the analysis is not spoilt.[1]

4. With fractional distillations the quantitative distillation of acetic or benzoic acid is ensured.

EXAMPLES

	No. of c.c. of distillate.	No. of c.c. N/100-NaOH used.
1st titration . . .	20 c.c.	6·00
2nd ,, . . .	15 c.c.	0·30
3rd ,, . . .	5 c.c.	0·05
4th ,, . . .	5 c.c.	0·02
Total . .	45 c.c.	6·37

Calculation

log (percentage of acetyl) = log (No. of c.c. of N/100-NaOH used) + log (factor) + 2 — log (mgm. of substance).

For acetyl, Factor = 0·4302 ; log Factor = $\bar{1}$·63370.
For benzoyl, Factor = 1·0504 ; log Factor = 0·02135.

Analytical Examples

Acetyl-glycine, $C_4H_7NO_3$ (mol. wt. 117·05).
Theory, 36·75 per cent. $COCH_3$.

mgm. of substance.	No. of c.c. N/100-NaOH used	Percentage of $COCH_3$ found.
5·604	4·74	36·57

(Saponified for two and a half hours with sodium hydroxide in methyl alcohol.)

Psicain, $C_{17}H_{21}O_4N . C_4H_6O_6$ (mol. wt. 453·2).
Theory, 23·18 per cent. COC_6H_5.

mgm. of substance.	No. of c.c. N/100-NaOH used	Percentage of C_6H_5CO found.
15·922	3·53	23·28

(Saponified for one hour with 5 N sodium hydroxide.)

[1] According to the course of the distillation, 99–99·5 per cent.

VIII. The Determination of Methyl Groups Attached to Carbon Method of R. Kuhn and H. Roth [1]

History and Principle of the Method

As early as 1888, J. Messinger [2] used chromic acid for the determination of carbon in the wet way in organic substances. However, because too low values were obtained with fatty acids, the oxidation products must still be led over ignited copper oxide. The complete combustion of fatty acids was attained by L. J. Simon [3] through oxidation with the addition of silver chromate. Meanwhile, this process has also been applied in microanalysis. [4]

With the method here described, which depends on the macro-process (Process B) worked out by R. Kuhn and F. L. Orsa, [5] the substance is heated in a sulphuric-chromic acid mixture under a reflux condenser. The conditions of oxidation are so chosen that the compounds containing methyl groups attached to carbon are decomposed with formation of acetic acid. Acetic or benzoic acid previously present is not affected by the oxidising agent. [6]

Liquids and also volatile products of decomposition of solids may, however, be withdrawn to the colder part of the apparatus even before they have been quantitatively oxidised. In more recent times, L. King has developed the oxidation in the microbomb for such substances. This method makes it possible to determine the methyl groups attached to carbon with the greatest accuracy, even in extremely volatile substances, such as ether.

After reduction of the excess of chromic acid, the acetic or benzoic acid is fractionally distilled from a solution of phosphoric acid and titrated.

As will be developed, p. 204, the amount of acetic acid formed by the oxidation of compounds containing methyl groups attached to carbon depends very largely on the further linkage of the $C—CH_3$ group.

Because ethoxyl groups give the theoretical yield of acetic acid, this method may be used for the determination of ethoxyl on the one hand and, on the other, to ascertain the sum of ethoxyl and acetyl present, *e.g.*, the di-ethyl ester of tetra-acetyl-mucic acid yields four acetyl and two ethoxyl groups.

If methoxyl and ethoxyl are to be differentiated, the sum of the alkoxyl groups is first determined as on p. 171, and afterwards the

[1] *Ber. dtsch. chem. Ges.*, **66**, 1274 (1933).
[2] *Ber. dtsch. chem. Ges.*, **21**, 2910 (1888); **23**, 276 (1890).
[3] *Compt. rend. Acad. Sci., Paris*, **170**, 514 (1920); **174**, 1706 (1922); **175**, 167, 525, 768, 1070 (1922); **176**, 1065 (1923); **177**, 265 (1923).
[4] *Cf.* p. 68.
[5] *Z. angew. Chem.*, **44**, 847 (1931).
[6] In a mixture of 4 c.c. of 5N-chromic acid and 1 c.c. of sulphuric acid, sp. gr. 1·84, there is no loss of acetic acid on heating for two hours under a reflux condenser.

ethyl groups are determined as acetic acid by oxidation with chromic acid.

As stated on p. 196, correct determinations of acetyl and benzoyl groups can sometimes only be made by this method.

Course of the Analysis

Because the analysis is carried out in the apparatus described. for the acetyl and benzoyl determination, reference will only here be made to the modifications of the method which are required for the oxidation with chromic acid. The reagents have already been given (p. 195).

Solids. After the solubility of the very finely powdered substance in the oxidation mixture has been ascertained,[1] the material is weighed, the flask clamped and the condenser attached. Then 5 c.c. of the oxidation mixture, prepared from 20 c.c. of 5N-chromic and 5 c.c. of sulphuric acid, sp. gr. 1·84, are added. (During mixing, the chromic and sulphuric acid must be cooled.) After the inlet tube has been connected and the funnel put on, the mixture is boiled for one and a half hours under a reflux condenser. The flask is heated in a small Babo funnel.

After the mixture has cooled, the glass rod S is removed, the condenser rinsed down with 5–7 c.c. of water, the oxygen gasholder is disconnected, and the condenser, the inlet tube (which has been rinsed), and the small funnel are removed.

Liquids. These, and also solids which are volatile or which form volatile decomposition products which escape from the oxidation mixture, are oxidised in a small pressure tube, 30 cm. long, as on p. 105. One brings the solid substance, as usual, into the pressure tube by means of the long-handled weighing tube (p. 79), and rinses down the residue adhering to the wall with the sulphuric chromic acid mixture, using 5 c.c. Liquids with low vapour pressure are introduced in the micro-weighing bottles, as on p. 106. Very volatile liquids, such as ether, are weighed by A. Pirsch's method (p. 242). For this the sulphuric-chromic acid mixture must be previously placed in the pressure tube and there cooled. If the mixture is warmer than the capillary, the liquid volatilises before the tube is sealed. The capillary is pushed point downwards into the oxidation mixture and the tube is fused together quickly as on p. 106. One with little experience in sealing should cool the tube during this manipulation also. The micro-pressure tube, in a furnace placed slanting (p. 105), is heated to 120° C. for one and a half hours. After cooling it is opened as on p. 107, the tip is broken off and rinsed over the oxidation flask with 1–2 c.c. of distilled water. The

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[1] Some anthocyanidines must first be dissolved in sulphuric acid; chromic acid is added drop by drop whilst cooling.

capillary in the pressure tube is then crushed with a glass rod and the mixture, with the fragments of glass, are rinsed quantitatively into the oxidation flask with about 6–8 c.c. of distilled water, rinsing the rod also.

For the reduction of the excess of chromic acid, dilute hydrazine solution is added drop by drop whilst keeping cool, to reduce the brown or dark-brown chromic-sulphuric acid mixture, till the first tinge of green is observed. An end point of pure green must be avoided. With further cooling, the sulphuric acid is neutralised with 6 c.c. of 5 N-sodium hydroxide and acidified with 1 c.c. of phosphoric acid (sp. gr. 1·7). Afterwards three to four pieces of pumice are placed in the flask, and the distillations and titrations proceed as in the acetyl determination.

Calculation

1 c.c. of $N/100$-NaOH corresponds to 0·15023 mgm. CH_3 or 0·60031 mgm. CH_3 . COOH.

$$\text{For } CH_3, \text{ log factor} \qquad = \bar{1}\cdot17676.$$
$$\text{For } CH_3 . COOH, \text{ log factor} = \bar{1}\cdot77838.$$

log (percentage of CH_3) = log (c.c. of $N/100$-NaOH) + log (factor) + 2 − log (mgm. of substance).

Examples :—

Acetyl-salicylic acid, $C_9H_8O_4$. Mol. wt., 180·03.

mgm. of substance.	No. of c.c. N/100-NaOH.	Percentage of CH_3 (to C).
13·718	7·66	8·38 Found.
		8·34 Theory.

Time of oxidation, 75 minutes.

Bixin, $C_{25}H_{30}O_4$. Mol. wt., 394·23.

mgm. of substance.	No. of c.c. N/100-NaOH.	Percentage of CH_3 (to C).
9·275	9·34	15·13 Found.
		15·24 Theory.

Time of oxidation, 60 minutes.

Ether, $C_4H_{10}O$. Mol. wt., 74·07.

mgm. of substance.	No. of c.c. N/100-NaOH.	Percentage of CH_3 (to C).
2·513	6·78	40·53 Found.
		40·54 Theory.

Time of oxidation at 120° C, in pressure tube, 90 minutes.

YIELD OF ACETIC ACID, AS PERCENTAGE OF THE THEORETICAL

C_2H_5 . OH	C_2H_5 . O . C_2H_5	C_2H_5O . CO . R
100	100	95–100
CH_3 . CO . OR	CH_3 . CO . CH_2 . R	CH_3 . CHOH . CHOH . R
100	85	95

CH_3 . CH = CH . R = CH . C(CH_3) = CH—

85 90

Compound.	Molecular fraction oxidised.	Acetic acid as percentage of theory.
Acetophenone . . .	0·1	10
m-Xylene . . .	0·24	12
1-3-Dimethyl-2-hydroxybenzene . .	1·1	55
Thymol	1·4	70
o-Methylamine . . .	0·7	
1-Hydroxy-4-methyl-2-benzoic acid . .	0·75	
p-Toluidine . . .	0·60	
m-Xylidine . . .	1·2	60
6, 8, 9-Trimethyl-isoalloxazine (a m-Xylene derivative)	1·40	70
6, 7, 9-Trimethyl-isoalloxazine . . .	0·9	45
N-Ethylaniline . . .	0·9	

Thus, for the " additivity " worked out : According to the above table, with α-ionone, there are to be expected 0·4 + 0·85 + 0·85 = 2·1 molecules of acetic acid per molecule. There were found 2·0 molecules.

It should here be emphasised that higher fatty acids are not always evenly decomposed to acetic acid. For examination, acid distilled from a larger weight in a second test is treated with p-bromophenyl-acylbromide and the acid is identified by the melting-point of the corresponding p-bromophenacylester.[1] Also, the melting-point of the sodium acetate[2] (m.p. 328° C.) may be used.

IX. The Determination of Isopropylidene Groups, by the Method of R. Kuhn and H. Roth[3]

Basis and Theory

With decomposition products of lycopine there arose the problem of undertaking the determination of isopropylidene groups on small amounts. This was solved with remarkable accuracy by observing a series of precautionary measures.

Isopropylidene groups which are bound to oxygen, as in acid compounds of sugar, ketonic acids, etc., Formula I, may be quantitatively decomposed by dilute acids and determined by the quantitative titration of acetone as iodoform.[4] Isopropylidene groups

[1] W. L. Judefind and E. E. Reid : *J. Am. Chem. Soc.*, **42**, 1043 (1920) ; and C. G. Moses and E. E. Reid : *J. Am. Chem. Soc.*, **54**, 2101 (1932).

[2] R. Kuhn, A. Winterstein, and L. Karlowitz : *Helvet. chim. Acta*, **12**, 64 (1929).

[3] *Ber. dtsch. chem. Ges.*, **65**, 1285 (1932).

[4] O. Svanberg and K. Sjöberg : *Ber. dtsch. chem. Ges.*, **56**, 1452 (1923). K. Freudenberg, A. Noe, and E. Knopf : *Ber. dtsch. chem. Ges.*, **60**, 238 (1927). A. Grün

attached to carbon, Formula II, are split off as acetone by means of azone. In this case aldehydes, which react similarly with hypoiodite, must be decomposed by permanganate[1] before distilling off the acetone for the titration.
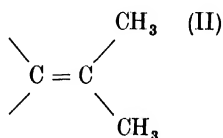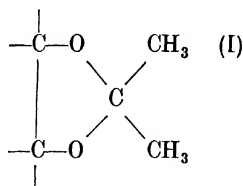
The course of the reaction follows the equations :—

$$3I_2 + 6KOH \rightarrow 3KI + 3KIO + 3H_2O \qquad (a)$$
$$CH_3 . CO . CH_3 + 3KIO \rightarrow CHI_3 + CH_3 . COOK + 2KOH \quad (b)$$
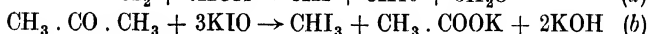
The published statements [2] on the conditions of reaction for the iodimetric determination of acetone are rich in contradictions. According to H. Elsner,[3] the temperature, time of action, and concentration should be of the greatest importance and occasionally cause much too high results ; these statements could not be confirmed. Appreciably excessive consumption of iodine is only observed if impure reagents are used. The degree of purity of the water must be given the greatest attention. Exact neutralisation of the alkali by sulphuric acid before the titration and the use of only 10–20 per cent. excess of iodine are also a source of error. It has been shown that one should acidify for the titration and should use 50–100 per cent. excess of iodine. The error in titration corresponding to the degree of dilution is, e.g., 0·04 c.c. of N/20 iodine for the addition of 200 c.c. of water ; it can therefore be neglected under the given conditions, in which 40 c.c. are added.

In numerous experiments it was established that the acetone formed is stable towards ozone and also towards permanganate acidified with acetic acid under the selected conditions of experiment.

and R. Limpächer : Ber. dtsch. chem. Ges., **59**, 695 (1926). K. Freudenberg, W. Dürr, and H. von Hochstetter : Ber. dtsch. chem. Ges., **61**, 1735 (1928). H. Elsner : Ber. dtsch. chem. Ges., **61**, 2364 (1928). A. Grün : Ber. dtsch. chem. Ges., **62**, 473 (1929).

[1] C. Harries : Ann., **343**, 311 (1906); **374**, 288 (1910) ; **410**, 8 (1915). V. Grignard, J. Doeuvre and R. Escourrou : C. R. Acad. Sci. Paris, **177**, 669 (1923) ; Bull. Soc. chim. France (4), **35**, 932 (1924). J. Doeuvre : Bull. Soc. chim. France (4), **39**, 1594 (1926). V. Grignard and J. Doeuvre : C. R. Acad. Sci. Paris, **187**, 270, 330 (1928) ; Bull. Soc. chim. France (4), **45**, 809 (1929). R. Escourrou : Bull. Soc. chim. France (4), **43**, 1088 (1928). P. Karrer, A. Helfenstein, B. Pieper and A. Wettstein : Helvet. chim. Acta, **14**, 435 (1931).

[2] J. Messinger : Ber. dtsch. chem. Ges., **21**, 3366 (1888). L. F. Goodwin ; J. Amer. Chem. Soc., **42**, 39 (1919). I. M. Kolthoff : " Die Massanalyse," Vol. 2, p. 430 Berlin, 1928. H. Elsner : Ber. dtsch. chem. Ges., **61**, 2364 (1928). " Klinische Mikromethoden zur Acetonbestimmung im Harn." M. Richter-Quittner : Biochem. Z., **93**, 163 (1919). H. Lax : Biochem. Z., **125**, 262 (1921). P. Rona : " Praktikum physiologischer Chemie," Vol. 2, p. 453f., Berlin, 1929.

[3] H. Elsner : Ber. dtsch. chem. Ges., **61**, 2364 (1928).

If consideration is given to these circumstances, then even with small amounts (2 or 3 mgm.) of acetone, the results only possess an absolute error of less than ± 0.5 per cent.

While isopropylidene groups attached to oxygen (I) may be determined with great accuracy, the theoretical amount of acetone is only obtained in a few cases after ozonisation of compounds in which these groups are attached to carbon (II). With all terpenes which are credited with "acetone groups," the acetone values are below those expected. Because the deficit is frequently covered by formic acid and formaldehyde, V. Grignard[1] especially came to the conclusion that mixtures in equilibrium of compounds containing CH_2 groups :—

$$CH_2 : C(CH_3)CH_2 \quad \alpha\text{-Form} \qquad \text{(III)}$$

and compounds containing the isopropylidene radicle

$$(CH_3)_2C : CH \qquad \beta\text{-Form} \qquad \text{(IV)}$$

are present. This conception is shared by J. L. Simonsen, who is one of the best authorities on terpene chemistry. He considers that even in beautifully crystallised compounds, which to all appearance are uniform, equal amounts of the α and β forms are assumed.

Because the absorption spectrum of dehydrogeranic acid[2] (m.p. 137° C.) obtained synthetically (V) approximates very closely to that of octatrienoic acid (VI), apparently only the isopropylidene, or β, form is present.[3]

$$H_3C \diagdown C = CH-CH = C(CH_3)-C = CH-COOH \qquad \text{(V)}$$
$$H_3C \diagup$$

$$H_3C-CH=CH-CH=CH-CH=CH-COOH \qquad \text{(VI)}$$
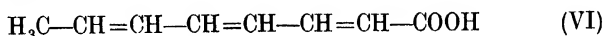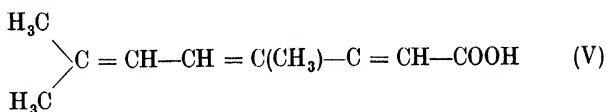
With the decomposition of (V) by ozone, as also from its sodium salt, only 0.60–0.65 molecule of acetone per molecule is obtained. According to Grignard, up to 0.35–0.40 molecule were to be inferred as the α-form. Because this conclusion, in the present case, does not agree well with that from the independent physical method—i.e., the absorption spectrum—one has to reckon further with the possibility that by the decomposition through oxidation with ozone or permanganate β-forms may partly yield decomposition products of the α type.

The acetone deficiency on decomposition of isopropylidene

[1] V. Grignard, J. Doeuvre and R. Escorrou: *Bull. Soc. chim. France* (4), **35**, 932 (1924).
[2] R. Kuhn and M. Hoffer: *Ber. dtsch. chem. Ges.*, **65**, 651 (1932). F. G. Fischer and K. Löwenberg: *Ann.*, **494**, 263 (1932).
[3] The spectrum of the α-dehydrogeranic acid must approach that of sorbic acid very closely.

compounds with ozone is probably due to reactions which are closely connected with the known rearrangement of acetone peroxide to form methyl acetate. A sure valuation of the results, to which the determination of these groups leads in its application to natural products, requires therefore numerous comparisons with the acetone
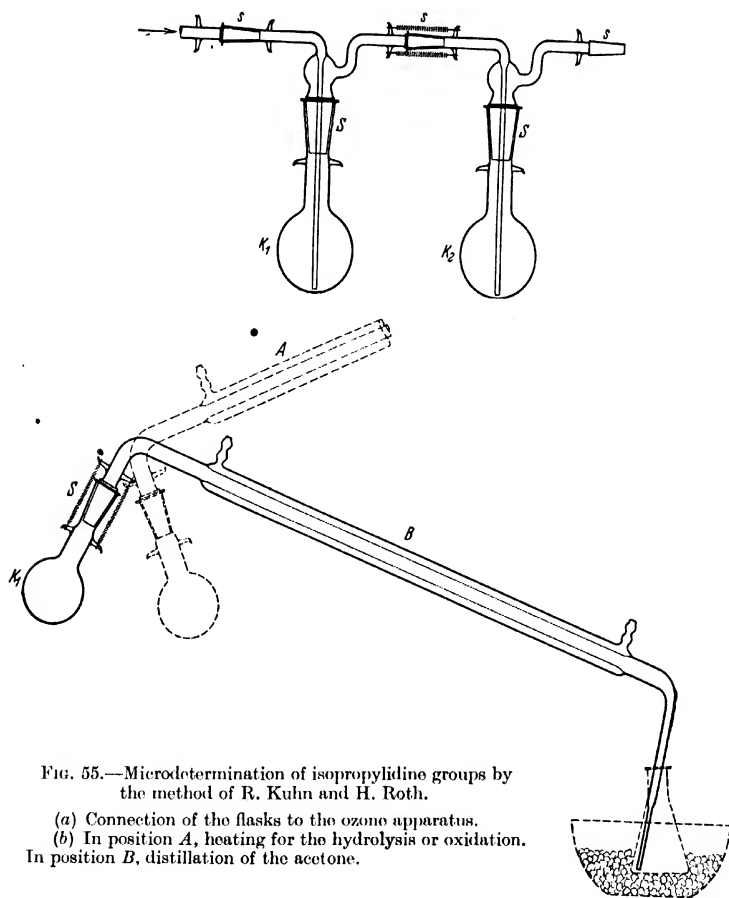
FIG. 55.—Microdetermination of isopropylidine groups by the method of R. Kuhn and H. Roth.

(a) Connection of the flasks to the ozone apparatus.
(b) In position *A*, heating for the hydrolysis or oxidation.
In position *B*, distillation of the acetone.

worked out on compounds of known constitution. In this, the process resembles that for the determination of methyl groups attached to carbon.

For the estimation of results, the following circumstances are important : As acetone resembles other methyl ketones in the quantitative formation of iodoform with hypoiodite, and as these are only decomposed to a small extent with permanganate it is necessary, in substances of unknown constitution, to determine the

acetone in a control test as acetone-*p*-nitrophenyl hydrazone, which is identified by its melting-point (149·5° C.).[1]

It is also to be noted that acetone is formed in considerable amounts from compounds which contain no isopropylidene groups, *e.g.*, thymol, terpin hydrate, isopropyl alcohol. Isopropyl groups in the neighbourhood of which hydroxyl and double bonds occur tend especially to form acetone.

Apparatus

The very simple apparatus consists of the ozone apparatus, two round-bottomed flasks with standard ground-in joints, and a condenser with a similar joint.

Any apparatus which is usual in laboratories may be used for the decomposition with ozone. It is connected with the inlet tube of the flask K_1, by the standard joint *s*.

The Jena glass flasks K_1 and K_2, of 100 c.c. capacity, are exactly alike and are provided with standard joints S (No. 2). In the corresponding stoppers there are the ozone inlet tubes, which end just above the bottom of the flasks, and the ozone delivery tube. Through *s* (No. 0) they are connected with the ozone apparatus and with one another. Strong steel springs attached to hooks press the joints, which have previously been moistened with meta-phosphoric acid, tightly together so that no gas leaks.

The Liebig condenser, American form, has a mantle 50 cm. long and possesses the standard hollow stopper S, which fits into the standard joint of the flask. The condenser is bent through 80 degrees at 6 cm. from S. In the position A it is used as a reflux condenser, in B it is used for distilling off the acetone without breaking the connection with the flask. To avoid loss of acetone at a second joint, the condenser is drawn out, at the other end, directly to an adaptor.

The Reagents [2]

Acetic Acid. E. Merck's 99–100 per cent. " Indifferent to chromic acid for the determination of Wij's iodine value."

Sodium Hydroxide. 2 N.

Sulphuric Acid. 2 N.

Sulphuric Acid. N.

Iodine Solution. N/20. Twelve grams of potassium iodide, free

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[1] For this, the first 5 c.c. of the distillate are mixed with 7 c.c. of the *p*-nitro-phenylhydrazine reagent, which is prepared by dissolving 0·07 gm. of *p*-nitro-phenylhydrazine in 7 c.c. of 50 per cent. aqueous acetic acid. The acetone-*p*-nitro-phenylhydrazone crystallises out in yellow needles. Limit of sensitivity, 0·004 per cent. If the solution is more dilute, a brownish substance is precipitated, which has not the melting-point of 149·5° C.

[2] Only water which has been distilled twice is used for reagents, rinsing and washing.

from iodate, are dissolved in a little water in a 1-litre graduated flask, 4·6 gm. of iodine are added and the solution made up to the mark ; the solution is kept in a brown bottle and the factor is determined after one day.

Sodium Thiosulphate Solution. N/20. Because this solution is not stable indefinitely and is used solely for titrating back the unused iodine, only a small stock is prepared.

Into a 250 c.c. graduated flask are pipetted 125 c.c. of exactly N/10 thiosulphate solution ; this is made up to the mark with boiled-out water free from carbon dioxide. In order to increase its stability, 0·1 gm. of sodium carbonate [1] is added and the solution is kept in a well-closed stock bottle. The solution can be standardised against N/20 potassium bichromate solution after one day.

Potassium Bichromate Solution. N/20. For the determination of the factor. With a calibrated pipette, 250 c.c. of standardised N/10 potassium bichromate solution are placed in a 500 c.c. graduated flask and diluted to the mark with water. After the solution has stood for twelve hours, it is checked by titration in duplicate against accurate N/10 sodium thiosulphate solution. Because the solution will keep unchanged for years, it is always ready for the determination of the factor of the N/20 thiosulphate solution.

Potassium Iodide Solution. Five per cent.

Starch Solution. One per cent. One gram of soluble starch is dissolved in 10 c.c. of cold water and afterwards poured into 90 c.c. of hot water.

Potassium Permanganate Solution. N. Sixteen grams in 500 c.c. of water.

Metaphosphoric Acid. Prepared from phosphorus pentoxide and a few drops of water.

The Determination of the Factors of the Solutions

Sodium Thiosulphate Solution. In a clean microburette of 10 c.c. capacity there is placed the standardised N/20 potassium bichromate solution and 7 c.c. are drawn off into a 100 c.c. Erlenmeyer flask with ground-in stopper. Twenty cubic centimetres of water are added, 2 c.c. of the colourless 5 per cent. potassium iodide are pipetted into it, the mixture is acidified with 5 c.c. of 2 N-sulphuric acid and allowed to stand for two minutes in the closed flask. Meanwhile, the N/20 sodium thiosulphate which is to be standardised is poured into a second microburette and the iodine liberated is then titrated by it, in drops which follow one another rapidly, to a pale brown ; 3–4 drops of starch solution are added and the solution similarly titrated till it is just decolorised. From

[1] Or, according to C. Meyer and E. Kerschbaum, 1 per cent. of its volume of amyl alcohol. *Z. analyt. Chem.,* **73**, 321 (1928).

the volume, read accurately to $\pm$ 0·01 c.c., the factor for this thiosulphate solution is calculated. If the factor in at least two titrations is obtained accurate to $\pm$ 0·2 per cent., the N/20 thiosulphate is now used to standardise the:

Iodine Solution. The determination is carried out as above, with the difference that the iodine solution, without potassium iodide, is used and titrated similarly with sodium thiosulphate after acidifying with sulphuric acid. The factor may then be calculated.

Because of its permanent stability the N/20 potassium bichromate solution is also suitable for the standardisation of N/30 and N/50 sodium thiosulphate. In order that not more than one burette full of thiosulphate may be used, only 6 or 3·5–3·8 c.c. respectively of the potassium chromate solution may be used.

The Course of the Analysis

1. *Hydrolysis*, when the isopropylidene group is attached to oxygen. As much of the material to be analysed as will form 1·5–2·5 mgm. of acetone is weighed, from the long-handled weighing tube of the nitrogen determination, into the clean dry flask K_1; 10 c.c. of N-sulphuric acid and a few pieces of pumice are then added, the condenser fitted well in at the joint after moistening with metaphosphoric acid, and the joint secured by hooking on the steel springs.

The condenser is now clamped in position A, the flask is protected by an asbestos wire gauze on a ring, and the water is turned on through the condenser. The solution is heated with a Bunsen burner so that it just boils. The acetone is usually split off after only a few minutes, but as a precaution the solution is boiled for ten to fifteen minutes. It is then allowed to cool.

2. *Ozone decomposition*, when the isopropylidene group is attached to carbon. The substance is placed at the bottom of the flask as described above and dissolved in 3 c.c. of 99–100 per cent. acetic acid. It is important that it should be completely dissolved; the solution may be warmed for this. Substances insoluble in acetic acid are very finely ground in an agate mortar and ozonised finely dispersed in the acetic acid.

To weigh oils and liquids, the micro-weighing bottles are used as on p. 106, or a small platinum boat is used. Liquids with high vapour pressure are weighed by J. Pirsch's method (p. 242), and the capillary is afterwards crushed with a glass rod in the acetic acid.

The ozone apparatus is first set in action and the speed of the oxygen current is regulated at 20 c.c. per minute. The Siemens and Halske A.-G. ozonisator, Model Oz, gives under these conditions oxygen containing 3·2 per cent. of ozone. The standard joint S of the flask K_1 is secured with a steel spring, after previously moistening

the inner part with metaphosphoric acid. In the second flask K_2, 3 c.c. of water are placed, the inner part inserted as above, the inlet tube (Fig. 55a) is connected with the delivery tube of the first flask, and the connections are secured with steel springs. The flask is then connected at s with the ozone apparatus and the second flask K_2 is cooled with melting ice. As a rule, the time required for ozonisation is from two to three hours.

The ozone apparatus is then disconnected and removed. After disconnecting the joints at S, the inlet tubes in both flasks are rinsed with about 10 c.c. of water and removed. The contents of K_2 are then rinsed with about 10 c.c. of distilled water into the acetic acid solution of K_1, the acid is neutralised with 16 c.c. of 2 N-sodium hydroxide, and 5 c.c. of N-potassium permanganate solution, with a few pieces of pumice, are placed in the flask. The condenser, the joint of which has been moistened with metaphosphoric acid, is inserted in the flask, the joint S secured, and the condenser is placed in position A and clamped. The solution is now boiled for ten minutes on the wire gauze, to decompose the ozonide and to oxidise the decomposition products which would affect the titration. Great care must be taken that the water is running freely in the condenser during this. After removing the flame, one ascertains whether some permanganate is still present. If all should have been used up, 5 c.c. more must be added after cooling and the heating repeated.

Distillation. The condenser and flask are then clamped in position B, without disconnecting. During the oxidation, 10 c.c. of water have been cooled in a stoppered 100 c.c. Erlenmeyer flask in melting ice, as in diagram. This flask is now placed under the condenser so that the adapter is not at first immersed in the receiver, because then the water present would rise in the condenser during the cooling of the distillation flask. Only when the acetone begins to distil is the adapter dipped into the receiver. A Babo funnel is used for the distillation. After 20 c.c. of distillate have been collected, the receiver is lowered, the adapter rinsed with 2–3 c.c. of water, and the Erlenmeyer flask closed.

Titration. The acetic acid content of the distillate corresponds to a maximum of 30 c.c. of N/10 sodium hydroxide. This distillate is immediately made alkaline with 5 c.c. of 2N sodium hydroxide for the iodoform reaction and mixed with 10 c.c. of N/20 iodine solution from the microburette. The iodine is added rapidly drop by drop. Because, in the cold, the formation of iodoform is slow, the closed flask is allowed to stand for fifteen minutes at room temperature whilst shaking frequently. To complete the determination one acidifies with 10 c.c. of 2N sulphuric acid and titrates the

unused iodine after two minutes with N/20 sodium thiosulphate solution, using starch as indicator.

Attention must, however, be paid to the checking of new reagents and those which have not been used for some time, and especially of new flasks, by an exactly similar titration, using twice-distilled water.

Calculation

Because 1 molecule of acetone uses up 6 atoms of iodine, 1 c.c. of N/20 iodine solution corresponds to 0·484 mgm. of acetone or 0·3505 mgm. C_3H_6.

$$\log 0·3505 = \bar{1}·54459$$

log (percentage of C_3H_6) = log (No. of c.c. N/20 iodine) + log (factor) + 2 − log (mgm. of substance).

Examples :—

(a) Isopropylidene attached to oxygen :—

Diacetone-mannose,[1] $C_{12}H_{20}O_6$; mol. wt.; 260·15.

Theory, 32·34 per cent. C_3H_6.

mgm. of substance.	No. of c.c. N/20-Iodine.	Percentage of C_3H_6 found.
8·148	7·47	32·12
7·752	7·01	31·69

(b) Isopropylidene attached to carbon.

Dimethyl-acrylic acid, $C_5H_8O_2$; mol. wt., 100·06.

Theory, 42·00 per cent. C_3H_6.

mgm. of substance.	No. of c.c. N/20-Iodine.	Percentage of C_3H_6 found.
7·439	8·30	39·10

X. The Determination of the Number of Double Bonds through Catalytic Microhydrogenation, by the Methods of R. Kuhn and E. F. Möller [2]

History and Principles of the Method

Even up to the present time, only a little work has appeared on the micro-determination of the number of double bonds in un-saturated organic compounds. The determination is made by means of hydrogen and platinum catalysts. Two principles underlie the methods used :—

1. The volumetric measurement of the consumption of hydrogen at constant pressure in a micro gas-burette.

2. The manometric measurement of the decrease in pressure at constant volume, due to the consumption of hydrogen ; the mano-

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[1] We thank Dr. O. Schmidt, Heidelberg, for the best preparation of this.

[2] *Z. angew. Chem.*, **47**, 145 ('1934).

meters used for this are based on the simple and differential
manometers supplied by O. Warburg [1] for investigations of meta-
bolism. The simple manometers measure the decrease in pressure
against the atmosphere and, in consequence of pressure fluctuations,
especially with measurements lasting over long periods, they are
only approximately accurate even when a control manometer is
used. The differential manometers, on the other hand, which
measure the decrease in pressure against a second vessel of approxi-
mately the same size readily permit higher accuracy for more than
one order of magnitude.

The first volumetric arrangement originated with J. C. Smith,[2]
who worked with an average of 2 mgm. of material, the same
amount of platinic oxide, and a 2 c.c. burette. If the corrections for
different solvents, which are determined by test experiments, are
taken into consideration, the maximal error should be about
± 1 per cent. This method has recently been considerably improved,
by K. H. Slotta and E. Blanke,[3] to an accuracy of 0·5 per cent.

Manometric methods have been frequently described. J. F.
Hyde and H. W. Sharp [4] recommend a somewhat troublesome
arrangement of a double manometer (measuring and control
thermometer combined). H. Kautsky and W. Baumeister [5] have
determined the rate of hydrogenation of aqueous methylene-blue
solutions with platinum adsorbents in an ordinary Warburg appa-
ratus. Also Warburg [6] himself carried out many hydrogenations in
simple manometers. H. Willstaedt [7] also recently reported on
micro-hydrogenation in simple Warburg manometers with the help
of several control manometers. The amounts of water determined
with the stated manometric methods lie, on an average, between
0·2 and 2·0 c.c. They are undoubtedly very suitable for the
determination of only a few double bonds, which are readily hydro-
genated. Their simple manipulation is a great advantage with
research on orientation and for multiple investigations. (Possibly
for finding out the correct conditions in hydrogenation of complicated
compounds.) Also, the simple manometers are proportionally very
cheap, frequently at hand in biochemical, physiological and other
medical institutes, and easily adapted for the purpose of hydro-
genation.

For the exact analytical determination of the number of double

[1] O. Warburg : " Uber den Stoffwechsel der Tumoren (Berlin, 1926). Also,
H. W. Knipping and P. Rona : 3. Part, " Praktikums der physiologischen Chemie,"
p. 199 (Berlin : J. Springer, 1928).
[2] *J. Biol. Chem.*, **96**, 35 (1932).
[3] *J. prakt. Chem.*, **143**, 3 (1935).
[4] *J. Amer. Chem. Soc.*, **52**, 3359 (1930).
[5] *Ber. dtsch. chem. Ges.*, **64**, 2446 (1931).
[6] *E.g.*, O. Warburg and W. Christian : *Biochem. Z.*, **266**, 387 (1933).
[7] *Ber. dtsch. chem. Ges.*, **68**, 333 (1935).   *

bonds of highly unsaturated or very slowly hydrogenated substances (with over ten double bonds, requiring longer than three to five hours), the accuracy of the simple manometric method is too low. K. Kuhn and E. F. Möller [1] therefore developed a method with a differential manometer, which measures the hydrogen used up by the substance against a comparison substance under exactly similar conditions. The accuracy attained is such that even after hydrogenation for a period of forty hours an error of $\pm$ 0·5 per cent. is not exceeded. This method is here described. Reference is specially made to some improvements, which with continual use are found essential. The apparatus described may also be used for direct manometric micro-hydrogenation of substances which contain only a few double bonds. For this, one places in the comparison vessel exactly the same amounts of solvent and catalyst as in the hydrogenation vessel, but without the comparison substance, and carries out the determination as on p. 218. The accuracy, however, is somewhat less, from $\pm$ 1–2 per cent.

Apparatus

The vessels G are of Duroglass. Each has a long wide ground-in joint (Figs. 56 [2] and 71) and two limbs, each of which makes an angle of 30 degrees with the axis down its centre. The limbs are about 6 mm. in diameter ; one, Ga, is sealed and the other opens into the horizontal trough, Gb, of the dimensions $20 \times 10 \times 70$ mm. The place of transition must be blown so that the overflow of liquid from the appendage Ga into the trough and *vice versâ* through tilting the whole apparatus through about 45 degrees may have free play. The joint fits into the cap S. To this cap is attached the displacement body Sa, which serves to decrease the large volume at S as much as possible. It is essential that the joint should be outside the vessel, otherwise the risk of contact between the solvent and the grease of the stopper during the transfer between the trough and the appendage is too great.

The hoods S are connected with the measuring capillaries Kb of the manometer through the connecting capillaries Ka, which are bent twice at right angles ; the manometer is fastened to a wooden board (Fig. 56). The board is inserted in the corresponding part in the eccentric rod of the shaking device by means of a bayonet-piece. The measuring capillaries Kb, which possess a scale from 0·0 to 30·0 cm., divided into millimetres, are connected with one

another to form the manometer through a U-shaped capillary, Kd. In the middle of this a capillary piece branches off to the joint Hc of the removable filling-tube R. The capillary piece slopes slightly downwards and is as short as possible. Finally, both measuring capillaries are connected with one another through the capillary Kc just above the mark 30, by means of the three-way cock Ha. The capillary Kc has a cross-section of 2 sq. mm., whilst all the rest possess one of only 1 sq. mm. The third way of the cock Ha leads into the outlet cock Hb, which communicates with the atmosphere. Both Ha and Hb are provided with borings of just 2 sq. mm. cross-section.

Calibration of the Apparatus

One cuts across the connecting capillary Ka just above the hood S and undertakes two calibrations. (1) That of the vessel G with hood S and the attached part of Ka, with water. (2) With mercury, the connecting capillaries Ka and measuring capillaries Kb, up to the line 15·0 cm., inclusive of the horizontal capillary piece Kc leading through the three-way cock Ha.
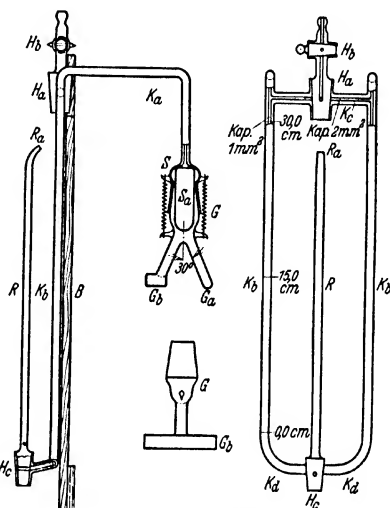
Fig. 56.—Micro-hydrogenation apparatus of R. Kuhn and E. F. Möller.

The first calibration is made as follows: The vessel G with the hood S is weighed empty, then S is taken off, G is filled to the top with water, and the hood replaced very quickly but with great care. The vessel with hood and the attached capillary must now be quite full of water.[1] A little water is then removed from the capillary, the position of the meniscus is marked, and the whole is weighed. After melting together the second calibration follows: With the apparatus inverted and the three-way cock Ha closed but not greased, mercury is poured through the hood S till the 15·0 mark of the water meniscus

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[1] Should this not be the case, the vessel G is warmed, with tapping, in a boiling water-bath and, after all the air has been driven out and water emerges from the capillary, water is sucked back through a bent capillary whilst cooling. With a correctly made hood S and some practice this is unnecessary; it is an advantage, in any case, to omit the heating, because during cooling the ground-in parts are easily pressed too tightly together and also other errors can appear.

is reached and the capillary piece *Kc* is filled through *Ha*, the level of the mercury in *Kb* is read, and the filled-up mercury is weighed. The difference up to the 15·0 cm. line is finally calculated from the cross-section of the capillary. The latter is similarly determined by calibrating by weighing with mercury. The accuracy of the calibration of the vessel is easily taken to $\pm$ 0·1 per cent., that of the capillary cross-section is somewhat lower.

Reagents

Hydrogen, electrolytically prepared by the I. G. Farbenindustrie A.-G., is very suitable ; one can also prepare it oneself, *e.g.*, in the Paneth [1] apparatus. To purify it from traces of hydrogen sulphide and oxygen, the hydrogen taken from a steel bottle by means of a reducing valve is led through a large spiral wash-bottle containing alkaline plumbite solution.[2] A tube containing granulated calcium-chloride serves for drying, and one with cotton-wool [3] holds back calcium chloride dust. A set of capillaries is inserted to regulate the current of hydrogen ; the pressure is measured by a mercury barometer. The apparatus for purifying and drying is connected by fusing together as far as possible, to avoid rubber connections. '

As solvents, *acetic acid* (Merck) and *alcohol* (Merck) may be used without further purification. *Hexahydrotoluene* (Schuchardt, for scientific purposes) must be shaken with sulphuric acid (Merck's, for forensic purposes), till it is practically stable in micro-tests against platinic oxide and hydrogen. The acid is renewed twice daily at first, then daily, next, every two days, and finally weekly. *Dekaline* is repeatedly shaken for short periods of time with Merck's oleum (5 per cent.). The acid is, however, somewhat coloured, even when the dekaline is already saturated towards platinic oxide and hydrogen. The hydrocarbons are finally carefully fractionated under diminished pressure. Recently *cyclohexane* (I. G. Farbenindustrie), purified like hexahydrotoluene, and also *chloroform* (Merck, p.a.), *tetrachlorethylene* (Merck puriss med.), *cyclohexanol* (Schering-Kahlbaum) and *octyl alcohol* (Fraenkel and Landau), which are subjected to hydrogenation with platinum-silica gel No. 17 (Membranfilter-Gesellschaft Göttingen), have been used successfully as solvents.

Platinic oxide and *palladium oxide* which one prepares oneself exactly according to the directions of R. Adams and R. L.

[1] Obtainable through Hanff and Buest, Berlin.
[2] Three to four grams of Merck's lead chloride are dissolved in 100 c.c. of 20 per cent. sodium hydroxide.
[3] The introduction of a tube with palladium asbestos, which is heated to a dull red-heat, has proved to be unnecessary. The last traces of oxygen are then reduced to water in the apparatus itself during the time in which the catalyst becomes saturated with hydrogen.'

Shriner,[1] and also the " *Tragerkatalysator* " (support-catalysts) of the Membranfilter-Gesellschaft m. b. H. Göttingen (platinum on silica gel).[2] For the profitable use of the individual catalysts, their concentrations, the solvents, and above all the mixtures of solvents, reference must most emphatically be made to the original [3] and to that of K. H. Slotta and E. Blanke.[4] It can here only be mentioned that it is very difficult to say in advance which conditions are suitable for a class of compounds or for a single compound, or even which are most favourable. With unknown substances, therefore, mostly only several hydrogenations under various conditions, possibly with the use of similar compounds, can lead to the goal.

Carrying out the Measurements

The purity of the substance to be hydrogenated is of the greatest importance. It is frequently observed that substances which give a perfect combustion can only be hydrogenated satisfactorily after further purification, which does not alter the values of the combustion.

Sorbic acid is almost exclusively used as comparison substance. The purification of the commercial product is done through crystallising from dilute alcohol and subliming in the high vacuum several times alternately. The preparation is kept in a high vacuum over tablets of potassium hydroxide.

The following are the requirements for a comparison substance : (1) Even with a very weak catalyst it must be hydrogenated with average velocity which is as uniform as possible. (2) It should show no increased absorption of hydrogen even with a very strong catalyst. The azobenzene proposed by H. Willstaedt [5] could therefore probably find application as calibration substance for the determination of the constants of the vessel, but not, however, as comparison substance in this process.

The weight of substance taken must be adjusted according to the consumption of hydrogen by the comparison substance, in order that too large pressure differences may not appear at the end of the hydrogenation. The initial velocity of hydrogenation of material and of the comparison substance should be of the same order of magnitude ; then the amount of substance may be so chosen that its consumption of hydrogen corresponds to up to 50 cm. difference of pressure. (This is thus more than the range which can be

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[1] *J. Amer. Chem. Soc.*, **45**, 1071 (1923) ; **46**, 1683 (1924). Naturally, for action on the substance, the oxides are not present as such, but only in the reduced state and saturated with hydrogen.

[2] R. Köppen : *Z. Elektrochem.*, **38**, 938 (1932).

[3] *Z. angew. Chem.*, **47**, 145 (1934).

[4] *J. prakt. Chem.*, **143**, 3 (1935).

[5] *Ber. dtsch. chem. Ges.*, **68**, 333 (1935).

measured ; in fact, only the difference in pressure is measured which corresponds to the difference in consumption of hydrogen by both substances. If, however, the initial speeds of hydrogenation of both substances differ too much, the amounts of substance taken must be correspondingly smaller, so that the initial pressure differences still fall in the measuring range. It is preferable, in such cases, frequently to omit the comparison substance. This may be done when thought advisable if only very small amounts of substance are at one's disposal. The further manipulation is not, however, altered through the omission of the comparison substance.

From 0·5 to 20 mgm. of the substance to be analysed and about 2–3 mgm. of sorbic acid are weighed in long-handled weighing-tubes and put in the appendages Ga of the vessels. The catalyst is weighed into the trough (with small amounts also with the micro-balance). Both vessels should be charged with the same amount as nearly as possible to ± 2 per cent. The solvent is then pipetted into the trough, using 1·50–3·00 c.c. (best, 2·00 c.c.). The joint is greased with viscous " apiezon " grease (type N),[1] inserted in the hood, and gripped with good spiral springs. The filling tube R,[2] which is freshly greased with " apiezon " grease before each measurement, is now placed so that it is connected with the capillary Kd through the joint Hc. The mouth Ra is joined by rubber tubing [3] to the system described above ; by this means the system may be alternately evacuated to about 20 mm. Hg and filled with the purified hydrogen. Previously the cock Ha is turned so that both vessels are in communication, the outlet cock Hb is, however, closed. During the evacuation the apparatus is strongly shaken by hand to remove all dissolved air. The evacuation and admission of the hydrogen are repeated about five or six times. After the last filling with hydrogen there should finally be a small excess pressure of about 50 mm. Hg in the vessels. Finally, the filling tube R is closed to Kd by turning through 180 degrees and the tubing is removed from its mouth. With readily volatile solvents, the vessels must be cooled in a suitable manner, because an alteration in volume through loss of solvent must not occur.

The apparatus is now placed in the thermostat at 25° C. (with a variation of not more than $\pm 0\cdot02°$ C.), and shaken strongly for about one hour. With support-catalysts, the time required for saturation with hydrogen is usually shorter, with platinum and palladium oxides it may be several hours. The shaking must be so vigorous that not only the solvent, but also the catalyst, is thrown

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[1] To be obtained through E. Leybold's successor, Köln. a Rh.

[2] Care must be taken that the narrow bore in the stopper is quite free from grease, in order that, later, the manometer liquid may easily pass through it.

[3] Pressure tubing treated with paraffin wax *in vacuo*.

strongly against the upper wall of the trough.   By quickly turning
the outlet cock *Hb* the excess pressure of hydrogen is let off, the
filling tube is now filled with an amount of Merck's absolute alcohol
which has been previously measured accurately, as manometer
liquid.   By very carefully opening, the alcohol is admitted through
Hc into the capillaries.   With correct construction and some care
no bubbles of gas enter the capillaries.   Afterwards the outlet cock
Hb is quickly opened and closed once more, so that the measuring
capillaries are filled just above the 15 line.   The exact adjustment is
finally made through removal of liquid by inserting a wad of
cotton-wool into the filling tube.   No appreciable change of pressure
against that of the atmosphere should now appear.   After this has
been ascertained, *R* is closed against *Kd* by turning through 180
degrees and sealed with mercury.   This is absolutely necessary,
because the alcohol slowly softens the grease.[1]   The three-way cock is
finally turned through 90 degrees so that the vessels are no longer in
communication and the apparatus can be used as a differential mano-
meter.   The final testing to constant pressure is then undertaken.

The apparatus is taken out of the thermostat and tilted in such a
way that the substance and comparison substance are transferred
from the appendage into contact with the catalyst in the trough.
The remainder of the substance is rinsed later into the trough by
fresh tilting.   The first reading is taken when the substance is
almost completely hydrogenated, which is usually the case in a short
time with sorbic acid.   The pressure differences in corresponding
intervals of time are then read, till constant pressure is reached
again.   The idea of constancy of pressure is naturally relative, and
the testing to constant pressure has to be adjusted according to the
rate of hydrogenation.

Calculation of the Number of Double Bonds

At the beginning of the experiment the pressures in both vessels
are the same, the difference of level $h = 0$; the volumes of gas V_x
and V_t, however, are different.   For the simplification of the
deduction it is assumed that the volume of hydrogen from the
x-substance is the larger and its rate of hydrogenation is much
lower, this is also practically the average case.   If the comparison
substance has now absorbed the volume a_t of hydrogen, by which
it should be completely hydrogenated, and if the pressures have

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[1] This was also the ground for the alteration of the cocks, in the apparatus here
described, from the original form.   If the alcohol previously poured in were evacuated
through the three-way cock, the grease partly dissolved in the alcohol smeared the
cock, and through want of grease in the stopper leakage all too readily occurs.
After the determination is finished, one lifts the filling tube out of the joint, without
tilting the apparatus, and lets the mercury flow off into a glass dish.

again been equalised, so that the difference of level $h = 0$, then the x-substance has absorbed the volume a'_x of hydrogen. Then the equation holds :—

$$\frac{a'_x}{V_x} = \frac{a_t}{V_t} \tag{1}$$

If the x-substance is now hydrogenated to the end, the difference of level h in relation to V_x is positive. If the volume of hydrogen which corresponds to this difference is called a_h, the sum of a'_x and a_h is equal to the volume a_x of the hydrogen absorbed by the x-substance at the end of its hydrogenation ; thus also

$$a'_x = a_x - a_h \tag{2}$$

Substituting for (2) in (1) :—

$$\frac{a_x - a_h}{V_x} = \frac{a_t}{V_t}$$

or

$$a_x = \frac{V_x \cdot a_t}{V_t} + a_h \tag{3}$$

The volume a absorbed stands to the molecular volume W of the hydrogen in the same ratio as the weight of material E to the molecular weight M of the substance to be hydrogenated ; $a : W = E : M$ for one double bond per molecule. If the substance has δ double bonds per molecule, than :—

$$\frac{a}{W} = \frac{E \cdot \delta}{M}, \text{ thus } a = \frac{W \cdot E \cdot \delta}{M}$$

$$a_t = \frac{W \cdot E_t \cdot \delta_t}{M_t}$$

$$a_x = \frac{W \cdot E_x \cdot \delta_x}{M_x}.$$

Substituting in (3) : —

$$\frac{W \cdot E_x \cdot \delta_x}{M_x} = \frac{V_x}{V_t} \cdot \frac{W \cdot E_t \cdot \delta_t}{M_t} + a_h,$$

and therefore :—

$$\delta_x = \frac{M_x}{E_x} \left[\frac{V_x}{V_t} \cdot \frac{E_t \cdot \delta_t}{M_t} + \frac{a_h}{W} \right].$$

There still remains a_h to be calculated from the difference of level h and the dimensions of the particular apparatus. If one points out that $\dfrac{a_h}{W}$ is a term for the correction and should only be 20 per cent. of the first term in the brackets, while the total pressure is not decreased at the end by more than 5 per cent. of the original, then one can substitute for a_h the expression for the volume absorbed

which was given by Warburg[1] for his differential manometer. V_x is then the "Test vessel," V_t the "Compensation vessel." Under the given conditions the accuracy of the approximate formula is within $\pm$ 0·3 per cent. Because, also, the absorption coefficients of hydrogen in the solvents used are very low and thus the amount of solvent is, at a maximum, 6 per cent. of the volume of gas, therefore the corresponding terms of the Warburg formula need not be considered. One then obtains the expression :—

$$\delta_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t}\cdot\frac{E_t\cdot\delta_t}{M_t}+\frac{h}{W}\left(1+\frac{A\cdot P}{2V_t}\right)\cdot\left(\frac{V_x}{P}\cdot\frac{273}{T}+\frac{A}{2}\cdot\frac{273}{T}\right)\right],$$

wherein A is the cross-section of the capillary, T the absolute temperature and P the normal pressure of the manometer liquid used, which one finally expresses by the specific gravity s; thereby, the formula may be still further reduced :—

$$\delta_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t}\cdot\frac{E_t\cdot\delta_t}{M_t}+h\cdot\frac{273}{T\cdot W}\left(1+\frac{A\cdot s}{2\cdot 1\cdot034\cdot V_t}\right)\right.$$
$$\left.\left(\frac{1\cdot034\cdot V_x}{s}+\frac{A}{2}\right)\right]\ .\ (4)$$

For negative values of h, also for the case when a_x is smaller than a_t, the expression for a_h must however be so altered that V_x and V_t are interchanged, because $V_t - h$ is now positive, V_t thus is now the "comparison vessel" and V_x the "compensation vessel" according to Warburg. The numerical alteration of the expression is not great, but with large values of h and large differences between V_x and V_t it falls in the order of magnitude of an appreciable percentage and must then be taken into consideration. The expression with negative values runs thus :—

$$\delta_x = \frac{M_x}{E_x}\left[\frac{V_x}{V_t}\cdot\frac{E_t\cdot\delta_t}{M_t}-h\cdot\frac{273}{T\cdot W}\left(1+\frac{A\cdot s}{2\cdot 1\cdot034\cdot V_x}\right)\right.$$
$$\left.\left(\frac{1\cdot034\cdot V_t}{s}+\frac{A}{2}\right)\right]\ .\ (5)$$

If one hydrogenates without the comparison substance, the first term in the brackets is zero, because $E_t = 0$, and :—

$$\delta_x = \frac{M_x}{E_x}\cdot h\cdot\frac{273}{T\cdot W}\left(1+\frac{A\cdot s}{2\cdot 1\cdot034\cdot V_t}\right)\left(\frac{1\cdot034\cdot V_x}{s}+\frac{A}{2}\right).$$

[1] O. Warburg; also W. Knipping and P. Rona. See footnote 1, p. 213. Warburg's expression runs :—

$$x = h\left[\left(1+\frac{\frac{A}{2}\cdot\frac{273}{T}}{\left[V'_g\cdot\frac{273}{T}+V'_F\cdot\alpha\right]\Big/P_0}\right)\left(\frac{V_g\cdot\frac{273}{T}+V_F\cdot\alpha}{P_0}+\frac{A}{2}\cdot\frac{273}{T}\right)\right].$$

Example :

Hydrogenation of very pure β-carotin with platinic oxide (10·1 mgm.) as catalyst and Dekaline and glacial acetic acid, 1 : 1, as solvent, using sorbic acid as comparison substance.

General conditions of experiment :—

$$T = 25 \cdot 00° \text{ C.} \pm 0 \cdot 02$$
$$W = 22504 \text{ c.c.}$$
$$s = 0 \cdot 789 \text{ (ethyl alcohol, } 18° \text{ C.}[1])$$
$$V_x = 33 \cdot 169 \text{ c.c.} - 2 \cdot 00 \text{ c.c.}[2] = 31 \cdot 169 \text{ c.c.}$$
$$V_t = 31 \cdot 618 \text{ c.c.} - 2 \cdot 00 \text{ c.c.}[2] = 29 \cdot 618 \text{ c.c.}$$
$$A = 0 \cdot 01016 \text{ c.c.}$$

Substituting these values in (4) and (5) :—

$$\delta_x = \frac{M_x}{E_x}\left[1 \cdot 052 \times \frac{E_t \cdot \delta_t}{M_t} + h \cdot \begin{cases} + \ 0 \cdot 001445. \\ - \ 0 \cdot 001373. \end{cases}\right]$$

when E is calculated in milligrams and h in centimetres.

Special conditions :—

$$E_x = 2 \cdot 342 \text{ mgm.} \quad M_x = 536 \cdot 4$$
$$E_t = 2 \cdot 338 \text{ mgm.} \quad M_t = 112 \cdot 06$$

After 3 minutes, $h = + \ 0 \cdot 15$ cm. $\delta_x = 10 \cdot 09$

,, $4\frac{1}{2}$ hours, $h = + \ 2 \cdot 65$ cm. $\delta_x = 10 \cdot 94$

,, 8 hours, $h = + \ 2 \cdot 65$ cm. $\delta_x = 10 \cdot 94$

$$\delta_x \begin{cases} \text{Calculated} = 11 \cdot 00 \\ \text{Found} \quad\quad = 10 \cdot 94 \end{cases}$$

D. THE DETERMINATION OF PHYSICAL CONSTANTS

I. The Determination of the Melting-point under the Microscope with the Melting-point Apparatus of L. Kofler and H. Hilbck [3]

It is sometimes necessary, besides determining the micro-boiling-point of a substance, to determine the melting-point also on still smaller amounts than those (about 1 mgm.) used in the usual melting-point tube. Thus, *e.g.*, in biochemical work or with chromatographic analysis, one sometimes hits upon extremely small amounts of substance, which cannot be obtained in weighable amounts, but which can frequently be recognised through the determination of their melting-points under the microscope, and by their absorption bands.

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[1] Room temperature.
[2] Volume of solvent.
[3] *Mikrochem.*, **9**, 38 (1931).

In the knowledge that microscopic determination of the melting-point not only saves material, but also makes it possible to observe the melting of the crystals most accurately, R. Kempf,[1] G. Klein,[2] and C. Weygand and W. Grüntzig [3] have already given heating stages for the microscope. If one places the material in very small apparatus on these heating stages, then, *e.g.*, sublimations and distillations may be carried out under continual observation through the eyepiece, under ordinary and also under diminished pressure.

All these stages are electrically heated, the temperature is read by an ordinary mercury thermometer, which is enclosed in the heater.

In the apparatus which will here be described, the temperature is not read as before by a mercury thermometer, but the potential determined by a thermo-element is read in a millivoltmeter calibrated in degrees.[4]

The Melting-point Apparatus

It consists of the heating stage (Fig. 72), a round metal box of about 85 mm. diameter and 15 mm. high. In this box there is the electric heating coil. Glass windows of 3 and 7 mm. diameter are placed exactly in the centre of the upper and lower walls. The heating stage stands on three " Eternit " feet and may be attached to any microscope by means of two adjustable clamps.

On the heating stage lies the hotplate, a nickel-plated copper plate, 25 mm. thick. It is removable and is fixed to the heating stage by two pegs. A hoop inserted sideways serves to remove the hotplate. The light passes from the mirror on to the preparation through a central boring of 1·5 mm. diameter. The temperature of the surface of the hotplate is measured with a calibrated copper-constantan element of the Physikalisch-Technischen Reichsanstalt, Berlin.[5] A nickel-plated copper plate, 4 mm. × 10 mm., is placed in a junction which is screwed on to the hotplate 20-30 mm. from the centre. Both wires of the element, in a length of 70-80 mm., are fixed to the hotplate by small screws—the copper wire directly, while the constantan wire is insulated from the support by means of small sheets of mica. The surface of the hotplate is secured from air currents by a detachable metal ring, 6 mm. high, which, with four side pieces, reaches about 5 mm. under the hotplate and is

[1] R. Kempf : " Die methoden der organischen Chemie," J. Houben, 3rd edition, Vol. I., p. 797, 1925.
[2] *Mikrochem.*, Pregl-Festschrift, p. 192, 1929.
[3] C. Weygand and W. Grüntzig : *Mikrochem.*, **10**, 1 (1932).
[4] Made by the optical works, C. Reichert, Vienna.
[5] On the use of other available measuring apparatus, see W. Jäger : " Handbuch der Elektrizität und des Magnetismus, Vol. I., p. 699.

protected by a removable glass plate. The metal ring possesses an asbestos covering on its outer wall. The glass plate is placed on this ring, the top of which is ground flat, and seals the space in the hotplate against outside air.

The free junction of the thermo-element is immersed in ice-water or in a thermos filled with ice-water, the temperature is checked on a projecting thermometer.

With this height of the metal ring and the glass plate which lies on it, magnifications up to about 135 may be obtained; only thicker objectives demand a greater approach. This magnification, however, usually suffices.

For the determination of the melting-point, the crystals are placed on a slide and brought exactly over the hole in the hotplate by pushing the slide; they are then covered with a cover-glass. Micro-sublimates on cover-glasses are so placed on the underlying slide that the cover-glass with the crystals underneath lies above the hole. In order to keep away air currents within the hotplate, two glass strips are placed at both sides of the cover-glass and on these a larger cover-glass or slide. Finally, the glass plate is placed on the metal ring.

As soon as the microscope has been focussed, the current is turned on through the hotplate and regulated with the help of the rheostat so that the millivoltmeter shows a rise in temperature of 2° C. With substances of which the melting-point is approximately known, the heating should be taken quickly up to 10°–15° C. below the melting-point.

According to the check analyses of the author the error below 200° C. is less than ± 1° C., and from 200°–300° C. it is at most ± 2° C. The average errors are lower.

Readily volatile substances must be cemented in by the method of G. Klein,[1] before the determination; this is done by edging round the cover-glass with cement which has been prepared from casein and milk of lime. According to L. Kofler, all commercial glass or porcelain cements may be used.

Cracks which appear on setting are closed with a new thin over-layer of cement. The cover-glasses should not be too thin, because in that case they warp too easily and thus no longer lie well on the slide, hence too high melting-points might be found.

To quote here the numerous methods of sublimation which have been published, would exceed the limits of this book. Reference should therefore be made to L. Kofler's [2] collected lectures in the sessions of the Deutschen Pharmazeutischen Gesellschaft, from

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[1] *Mikrochem.*, Pregl-Festschrift, p. 102 (1929).
[2] *Arch. Pharmaz.*, **270**, 293 (1932).

which one can see which process should be used for micro-sublimation in special cases.

II. The Determination of the Boiling-point on Small Amounts of Material, on the Principle of A. Schleiermacher [1]

Principle

It often happens that the organic chemist must give up boiling-point determinations, when only a few milligrams of the substance are available, because the usual determination, by distillation, even with the most modern apparatus, is inaccurate even with one hundred times the amount. In the boiling-point apparatus of A. Schleiermacher, illustrated on p. 226 (Fig. 57), the boiling-point of both liquid and solid substances can be determined on 2–5 mgm., in the range of 30°–250° C., to ± 0·1° C. without any correction ; this is done by means of a massive aluminium heating block made for the purpose and a short-range thermometer. The method requires no particular gadgets, and can quickly be made effective for the determination of the boiling-point in any laboratory. I have to thank Mr. H. W. Rzeppa for energetic assistance and for carrying out numerous boiling-point determinations.

Principle : The substance enclosed over mercury is heated to that temperature at which its vapour pressure is equal to that of the atmosphere at the time. The temperature thus determined is the boiling-point.

The method also allows the boiling-point to be determined in a simple manner at 760 mm. pressure, even if the current barometric pressure is different. Thus, if the height of the barometer is 750 mm. one warms further, till the vapour pressure has increased by 10 mm., as shown by the difference of the mercury levels. Then the vapour pressure is exactly 760 mm. and the temperature read corresponds to the normal boiling-point.

This method is limited to substances which are not decomposed by mercury at their boiling-points ; further, they can only be used up to temperatures at which the vapour pressure of mercury is negligible. Up to 250° C., the boiling-point of benzoic acid, no influence of the vapour pressure of mercury was observed.

It is necessary for the success of this method that the space above the mercury should be saturated with the vapour of the substance, *i.e.*, some of the material should still be liquid. The dimensions of the apparatus are such that, *e.g.*, with water 1–2 mgm. are found to be sufficient. After successive boiling-point determinations the substance can be recovered.

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[1] *Ber. dtsch. chem. Ges.*, **24**, 944 (1891).

The Apparatus

One either makes the boiling-point tubes oneself individually or obtains a stock of several at a time from the glass blower. An ordinary glass tube, 30 mm. long, of inner diameter 6–8 mm., is drawn out with the blowpipe or a strong Bunsen burner, after 10–12 cm., to a tube about 25 cm. long and 2·5–3 mm. bore. After about 18 cm. the narrower tube is drawn out once more to a thin-walled capillary 1–1·5 mm. bore and this is cut off after 2–3 cm. At the junction the capillary is finally drawn out with the micro-burner to a 4–5 cm. long hair-fine capillary (Fig. 57 (*b*)). The tube is now so bent to a U-tube (Fig. 57 (*a*)) that the wide limb is about twice as long as that provided with the capillary. For this, the walls of the tube are allowed to fall together in the flame, till the bore is 1·5–2 mm., and the tube is bent round.[1] The parallel limbs should almost touch one another at the top. The tube is carefully cleaned with warm sulphuric-chromic acid, water, and distilled water, and dried in the drying-oven at 110° C.

As may be seen from the illustration, the aluminium heating block consists of a massive block, of 90 mm. × 65 mm. surface and 170 mm. high. Exactly in the centre a cylinder of 28 mm. diameter and 140 mm. high is hollowed out, in which the boiling-point tube is placed during the determination. At the side there is a boring for an ordinary thermometer. In the broader front and back surfaces two large mica windows are placed, through which the level of the mercury can easily be observed. The mica plates, in a brass frame, are screwed fast to the aluminium heating block by means of six screws. When, in the course of time, they have become cloudy, they can be replaced by new ones.
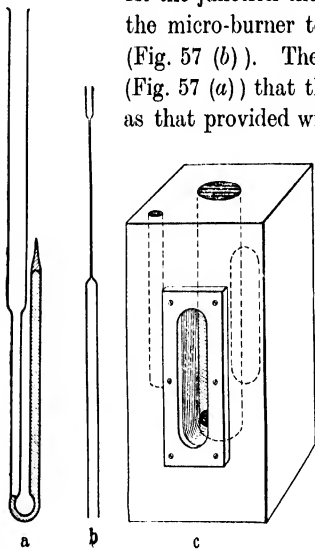
In order to obtain good constancy of temperature, the whole height of the aluminium block is surrounded with asbestos board, 2–3 mm. thick, which is made fast with a wire. On the upper surface one similarly places an asbestos board, in which a hole is bored with a cork-borer for the melting-point tube and another for the thermometer. As the preliminary test showed, the covering

FIG. 57.

a   b   c

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[1] If the bore is narrower, the adherence of the mercury becomes noticeable.

of the cylinder with asbestos is essential in order to have the same temperature in the whole range of the boring. The heating block is heated on a tripod with an ordinary burner.

For reading the boiling temperature a set of short-range thermometers is used, as in K. Rast's determination of molecular weight.

The Course of the Analysis

The boiling-point tube is filled with liquid as follows :—

One or two drops are placed as near as possible to the bend of the tube by means of a long glass tube which is drawn out to a point. By bending the boiling-point tube the liquid is allowed to slide just above the lowest point into the shorter limb. Solids (3–5 mgm.) are dropped with a spatula through the long limb and put in the same position as liquids by tapping. One then allows pure dry mercury to flow in, preferably from a dropping pipette, until it is about 10 mm. below the capillaries in both limbs. While liquids thereby collect on the surface of the mercury, with solids one must occasionally take care lest particles adhere to the walls of the right limb. They are easily brought on to the surface of the mercury by carefully warming the limb to the melting-point and carefully tapping it. If, however, one or another of the particles still remains in the limb, the determination is not affected.

One now places the tube in the heating block (without asbestos cover) and warms until the substance begins to boil gently. The air absorbed in it or in the wall of the tube then escapes through the capillary. Mercury is now added through the long limb till the upper end of the short limb is filled to the fine capillary with the liquid or liquefiable material, and then the fine capillary is sealed above the junction with the largest flame of a micro-burner. With correct sealing, which requires some practice, a quite small air-bubble remains behind (possibly also a small bubble of a gaseous decomposition product), which has no influence on the accuracy of the analysis—on the contrary, it prevents superheating. Finally, by lowering the boiling-point tube to the horizontal position, the mercury is poured out from the long limb up to the beginning of the bend (Fig. 57 (*a*)). The constriction at the bend prevents the escape of too much mercury and the appearance of an air-bubble in the now closed limb.

After working with the long thermometer, one now moves the corresponding short range thermometer up to the constriction in the wide limb of the glass tube, about 30 mm. below the substance, pushes the long limb through the hole in the asbestos board and allows the boiling-point tube to hang freely in the bore so that the bend is 10 mm. above the bottom of the hollow cylinder and the

substance about 40 mm. below the asbestos board. The air space between the inner wall of the limb and the thermometer is so small that practically no air current occurs, which would make a correction necessary. If thermometers of less than 4 mm. diameter are used, glass tubes of 5–6 mm. bore are suitable for the preparation of the boiling-point tube.

The heating of the block is adjusted according to the temperature required ; in any case, immediately the first bubble of gas is formed, the flame must be so adjusted that the mercury only sinks slowly in the closed limb. When both mercury columns·are exactly the same height, the temperature is read. This gives the boiling-point for the current barometric pressure. The boiling-point thus determined is best checked by at least three more readings. For this, one moves the mercury columns now in the one and then in the other direction by alternately raising and lowering the temperature of the heating block slightly and reads the temperature immediately the columns stand at the same level. The mean value of these readings is taken.

If the determination of the normal boiling-point (at 760 mm.) is required, the temperature is raised until the difference of level of the mercury in the open and closed limbs is equal to the depth of the barometer, at the time, below 760 mm. The vapour pressure

Comparative Boiling-point Determination

Substance.	Boiling-point[1] at 760 mm. Degrees.	Boiling-point[2] Found. Degrees.	Barometer Height. Degrees.
Ether	35·0	34·5	748
Water	100·0	100·1	760
Toluene	110·0	110·4	760
Pyridine	114·2	114·5	758
Phenol	181·0	180·5	752
		180·8	760
Dekaline	188·0	187·8	742
		188·2	760
Naphthalene	218·0	217·2	742
Benzoic acid	249·0	249·0	753
		249·2	760

in the closed limb is then 760 mm. It is sufficient to estimate the difference of level with the help of marks on the mica window or on the boiling-point tube, five millimetres apart, because a pressure difference of 1 mm. of mercury usually corresponds to a temperature difference of only about 0·04° C.

[1] Taken from the Chemiker-Kalender.
[2] Mean value of three to five readings.

III. The Determination of Molecular Weight

Determination from Raising of the Boiling-point
(Ebullioscopic Method)

Pregl's Method

The method does not differ in principle from the macro-method ; its development for micro-determinations began in 1912. After the excellent short-range Beckmann thermo-meter and the corresponding small boil-ing-vessel with inner condenser could be manufactured by the firm of Siebert and Kühn in Kassel, the conditions for the success of the analysis were satisfied because besides the small amounts of substance the amount of solvent used was correspondingly decreased. With 1·5 c.c. of liquid, 3 gm. of small platinum tetrahedra are placed in the boiling vessel, in order to surround the small bulb of 'the thermometer completely with liquid. It was impossible to maintain a constant boiling-point in these experi-ments by the methods previously em-ployed for boiling the liquid. This was only achieved by departing from the principle of heating in stagnant air, and so altering the conditions that air in uniform motion was warmed and passed around the boiling-vessel ; in this manner a steady temperature would be main-tained for several minutes. The founda-tions were thus laid for the construction of an apparatus, by means of which the determination of the molecular weight could be carried out by one introduction of 7–10 mgm. of material into 1·5 c.c. of solvent.

Fig. 58.—Apparatus for the determination of molecular weight by the rise of boiling-point.

During his first experiments F. Pregl had the advantage of the co-operation of Dr. H. Lieb. Concentric glass cylinders and tubes were used in order to prevent convection of air, and to ensure that the moving air current traversed a definite path.

The apparatus now used, the complete view of which is shown in Fig. 58 and the section in Fig. 59, consists essentially of a stand in which a rod can be clamped and vertically adjusted. The rod is
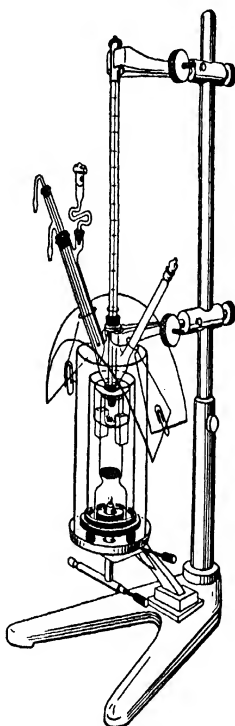
provided with two clamps; of which the lower holds the boiling-vessel and the upper the Beckmann thermometer which stands in that vessel. This arrangement permits these two most important parts of the apparatus to be brought into the heating chamber and to be removed from it without altering their relative positions.

The heating chamber is supported on a brass plate T 90 mm. in diameter; this is centrally perforated, and is held by a bracket to the foot of the stand. A circular ebonite stand with three feet, also supplied with a central perforation, is screwed to this metal plate. On its upper surface there are three concentric ridges. These ridges serve the purpose of maintaining two cylinders, Cy_2 and Cy_3, in a concentric position, the larger outer cylinder Cy_1 being supported by the metal plate T. The arrangement of these three cylinders will be clear from the accompanying illustrations. The outer cylinder is 140 mm. high and 84 mm. in diameter; the second, 120 mm. high and 48 mm. in diameter, while the third, innermost, cylinder consists of a shortened lamp cylinder, 110 mm. high, 36 mm. wide at the base and 26 mm. wide in the upper part. A circular disc of copper or brass gauze, Dr, is supported by the constricted portion, and serves to warm uniformly the whole cross section of the ascending air current at this point. A fourth simple cylinder, Cy_4, 26 mm. high and 36 mm. wide, surrounds the upper end of cylinder Cy_3, being held by three strips of asbestos board, which are interposed between these two cylinders. A mica plate is cemented to its upper opening with water-glass. A central perforation, of 16 mm. diameter, is cut into this plate with a suitable cork-borer, and that part of the boiling-vessel which is to be heated fits tightly into this perforation.

Fig. 59.—Apparatus for the determination of the molecular weight by the rise of boiling-point. (Section.)

T, Metal plate. M. Micro-burner. Cy_1, Cy_2, Cy_3, Cy_4, Glass cylinders, with mica plate cemented to Cy_4. Dr, Wire gauze.

A micro-burner can be inserted through the central perforations of the metal plate and the ebonite stand from below. This is capable of delicate regulation and produces a non-luminous flame about 15 mm. below the wire gauze.

When assembling the apparatus the short fourth cylinder Cy_4 is first slipped over the lamp cylinder, and adjusted with the aid of the

three asbestos strips, so that the mica plate is at a height of about
8–10 mm. above the lamp cylinder. After placing all the cylinders
in position it is advisable to light the flame at once, in order to heat
up the glass cylinders whilst the tablets are being prepared and
weighed. To carry out a determination, the boiling-vessel, which
has previously been washed with sulphuric-chromic acid, water and
alcohol and dried in a current of warm air, is inserted into the lower
clamp ; the platinum tetrahedra, which have been ignited imme-
diately beforehand, are added, and the Beckmann thermometer
inserted with the help of a well-fitting cork, so that the mercury
bulb reaches almost to the tetrahedra without touching them, its
upper end being held by the upper clamp. The upper clamp is then
loosened, the Beckmann thermometer removed, and 1·5 c.c. of the
solvent run on to the platinum tetrahedra from a weighed pipette.
The vessel is then closed at once by re-inserting the thermometer,
and the cooling water is turned on through the condenser. By
adjusting the rod, to which both clamps are attached, the bottom
of the boiling-vessel, which has so far been held at a high point to
one side of the ascending current of hot air, is now brought over the
central perforation of the lower disc and then lowered until the
boiling-vessel is surrounded by the mica disc at the level of the
surface of the liquid. On further lowering the boiling-vessel, the
fourth cylinder is forced downwards slightly over the third.

The path traversed by the hot air issuing from the apparatus is
clearly indicated in Fig. 59, from which it is also seen how the whole
apparatus is fully protected from above against air currents by a
plate of transparent celluloid. This consists of a rectangular sheet
200 mm. long and 130 mm. wide, in the central line of which three
round holes are cut with a cork-borer, at intervals of 35 mm., to fit
the three upper extensions of the boiling-vessel. In order to enable
this celluloid cover to be placed over the apparatus it has three
slits, which reach from the middle of its narrow edges to the two
side-holes, and from one of its longer edges to the central hole. After
the cover has been placed in position the edges of the two ends are
superimposed and held in position with ordinary letter clips, as
shown in the figure.

A number of conditions must be observed in order to ensure
regular boiling. The flame must always be so regulated that the
tap of the micro-burner is turned on to the fullest extent, and
the main cock is then turned down to such a point that the
micro-flame becomes quite small. Secondly, it is advisable to
introduce a pinchcock at a point in the rubber tubing, and
preferably to introduce a small piece of cotton-wool or some pieces
of string into the tubing under the pinchcock. The pinchcock is

then closed to such an extent that the size of the micro-flame is further reduced. Finally, the fine screw regulator of the micro-burner is adjusted in order to further reduce the flame by a barely perceptible amount. A small pressure regulator similar to that employed in the determination of carbon and hydrogen may be also introduced into the circuit; this differs from the other in that the long central tube is omitted.

The internal condenser is very important for the maintenance of constant ebullition, for if the condensed solvent falls back drop by drop the temperature falls as each drop is returned. This occurrence is particularly disturbing in the case of solvents of high boiling-point.
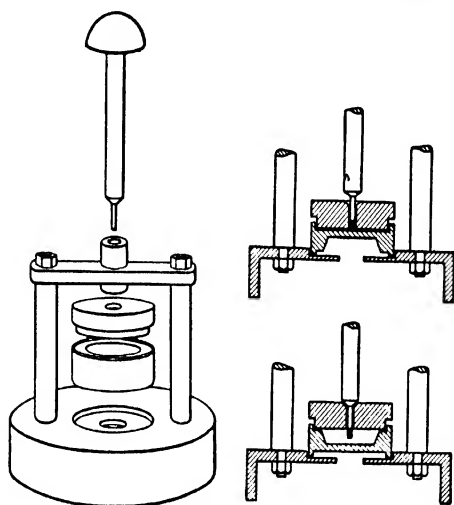
Fig. 60.—Tablet press with guide.

If, therefore, the lower point of the internal condenser has much play within the apparatus its position will be altered each time the latter is touched, and, therefore, the continuous return flow of the condensed liquid may be broken. Any such interference with the flow of the returning liquid may be avoided by sealing glass beads to the point of the condenser or, still better, by sealing a short length of platinum wire to its lower point.

The apparatus is so well protected from air currents that violent movements of a cloth in the immediate neighbourhood cause no change in the temperature registered by the thermometer. This is influenced, on the other hand, by illumination by direct sunlight, particularly if this is intermittent. The determination is therefore always carried out so that the apparatus is protected from direct sunlight.

An interval of fifteen minutes is usually required after the liquid

has begun to boil until the mercury thread takes up a steady
position, as observed by the lens which is also used with the micro-
azotometer. This is due to the fact that the glass of the cylinder
requires this amount of time in order to become uniformly heated.
Experience has shown that a constant temperature is more quickly
attained, and more accurate values obtained, by boiling
fairly vigorously. Before each reading the tendency of the
mercury to stick is overcome by gentle tapping with a
glass rod covered with a piece of rubber tubing, or by
alternate tapping with two fingers. After the mercury
column has remained steady within 0·002° C. during
several minutes, the substance may be introduced in the
form of weighed tablets.

It was necessary to construct a suitable tablet press
for the production of small tablets. The press illustrated
in Fig. 60 was constructed by Mr. A. Orthofer, Mechani-
cian at the Medico-Chemical Institute of Gratz Uni-
versity. It consists of a thick base plate, which has an
opening in the centre in which both the plates illustrated
fit. Two supports for the guide are also placed in it. The
upper plate of steel, 9 mm. thick, has a central perforation
of 2 mm. diameter, the top of which has a funnel-shaped
expansion. The lower part is hollowed out cylindrically
by turning, twice on the one side and once on the other.
As may be seen from the top right-hand illustration
(Fig. 43), if the plate with the funnel-shaped expansion is
placed on the other, its central perforation is closed by the
planed surface of the second plate. The substance is easily
inserted in the bore-hole through this expansion; after
this, the piston of the compressor, which exactly fits
into the hole, is pressed gently. Finally, a coherent
tablet is formed by pressing on the handle of the com-
pressor. In order to remove the tablet, the lower plate
is inverted (Fig. 60, bottom right-hand), and the former
is pressed into the hollow of the lower part by a sharp
blow on the compressor.

Fig. 61.—
Charging
tube (¾
actual
size).

In order to ·prevent the tablet from hanging in the side-tube it is
advisable to place it after weighing in a small tube, of 3-4 mm.
diameter and 15 mm. long, the closed end of which is provided with
a small side pit, and into which a glass thread about 150 mm. long
and 1 mm. thick is fused (Fig. 61). This is introduced as far as
possible into the side-tube, and the tablet released by rotation.

It was found by experience that it was advisable to produce
tablets in this manner of a weight of ʹ7 or, at most, 10 mgm. They

are weighed with an accuracy of 0·01 mgm. only, in weighing tubes such as are used for the determination of nitrogen (p. 79). The first tablet is introduced into the apparatus as soon as the boiling-point has become constant. After dissolution has occurred, the boiling-point rises gradually at first, then quickly, and finally acquires a correct value within two to three minutes at most. The second tablet is then introduced, so that the actual determination of the molecular weight of the material, introduced in two portions, takes five to six minutes at most, if all the conditions are correctly observed.

As the recovery of the dissolved substance after such a determination offers no difficulties, it is usually possible, after recrystallisation, to have 12–15 mgm. of material at one's disposal.

The molecular weight is calculated according to the formula :—

$$M = 100 \cdot \frac{K}{L} \cdot \frac{S}{\varDelta_t}, \text{ where}$$

S = Weight of substance.
K = Ebullioscopic constant of the solvent.
L = Weight of solvent.
$\varDelta_t$ = Observed raising of boiling-point.

TABLE FOR THE CALCULATION OF THE MOLECULAR WEIGHT, USING 1·50 C.C. OF SOLVENT

Solvent.	L (in gms.).	K [1] (in °C.).	$\frac{K}{L}$ (in °C/gm.).	$\log \frac{K}{L}$.
Ethyl ether. B.p. 34·6° C., $d_{20} = 0·714$	1·071	21·6	20·17	1·30466
Acetone. B.p. 56·1° C., $d_{20} = 0·792$	1·188	17·25	14·52	1·10197
Chloroform. B.p. 61·2° C., $d_{20} = 1·488$	2·232	38·8	17·39	1·24014
Ethyl alcohol. B.p. 78·3° C., $d_{20} = 0·789$	1·183	12·0	10·14	1·00002
Benzene. B.p. 80·5° C., $d_{20} = 0·879$	1·318	25·7	19·50	1·29001
Water. B.p. 100° C., $d_{20} = 0·998$	1·497	05·2	3·474	0·54078
Glacial acetic acid. B.p. 118·1° C., $d_{20} = 1·049$	1·573	30·7	19·52	1·29041

Examples :—
  Ethyl Alcohol as Solvent.

$$L = 1·175 \text{ gm.}$$
$$K = 12·0° \text{ C.}$$

Naphthalene, $C_{10}H_8$. Mol. wt., 128·06.

$$S = 14·44 \text{ mgm.}$$
$$\varDelta_t = 0·112° \text{ C.}$$
$$M = 131·7$$

  Benzene as Solvent.

$$L = 1·34 \text{ gm.}$$
$$K = 25·7$$

[1] For further ebullioscopic constants, see Landolt-Börnstein, 5th edition, Vol. 2, p. 1434, 1923.

Azobenzene, $C_{12}H_6N_2$. *Mol. wt.,* 182·07.

$$S_1 \;=\; 9\!\cdot\!67 \text{ mgm.} \qquad\qquad S_2 \;=\; 22\!\cdot\!57 \text{ gm.}$$
$$\Delta_{t1} \;=\; 0\!\cdot\!102^\circ \text{ C.} \qquad\qquad \Delta_{t2} \;=\; 0\!\cdot\!245^\circ \text{ C.}$$
$$M_1 \;=\; 181\!\cdot\!8 \qquad\qquad\qquad M_2 \;=\; 176\!\cdot\!7$$

Glacial Acetic Acid as Solvent.

$$L \;=\; 1\!\cdot\!598 \text{ gm.}$$
$$K \;=\; 30\!\cdot\!7$$

Anthraquinone, $C_{14}H_8O_2$. *Mol. wt.,* 208·06.

$$S \;=\; 11\!\cdot\!68 \text{ mgm.}$$
$$\Delta_t \;=\; 0\!\cdot\!107^\circ \text{ C.}$$
$$M \;=\; 209\!\cdot\!7$$

Determination of Molecular Weight by the Process of A. Rieche [1]

While the above-described process is preferable when using solvents of low boiling-point, such as ether, acetone or alcohol, the apparatus of A. Rieche (Fig. 62) is more suitable for solvents of higher boiling-point, such as benzene, water, pyridine or glacial acetic acid, although it entails the use of 4 c.c. of solvent and, consequently, of 15–25 mgm. of the substance. Even a transitory overheating of the Beckmann thermometer is almost excluded in this case, as it is always surrounded by a mixture of the boiling liquid and of its vapours. The liquid, which is boiled in the flask K (Fig. 62), is continually projected, together with its vapour, on to the thermometer through the nozzle D; the vapour condenses in the condenser, whilst the liquid falls back into the boiling-flask through the tube F. This tube is provided with a small baffle-cone

F I G. 6 2. — Rieche's apparatus for molecular weight determination.

B, which prevents the liquid from passing in the reverse direction. The liquid is heated over an asbestos-covered wire gauze by means of a micro-burner. Superheating is avoided by the use of platinum tetrahedra weighing 0·3 gm., and the apparatus is protected against

[1] *Ber. dtsch. chem. Ges.,* **59**, 218 (1926).

air currents by a simple cardboard cylinder. As in this apparatus a certain proportion of the substance is always present in the vapour form, the results are usually slightly low, but never by more than 5 per cent.

In order to carry out a determination, the platinum tetrahedra are inserted in the cleaned and dried apparatus, after which the baffle-cone is introduced and a thermometer also inserted by means of a tight-fitting cork, and carefully adjusted so that the bulb is completely covered by the inner tubular cylinder R, in which *position its lowest point is* about 5 cm. below the upper rim of the apparatus. The whole assemblage is then clamped into a stand above the asbestos-covered wire gauze and surrounded by a vertical cardboard cylinder. By means of a tared pipette 4 c.c. of solvent are added through the side-tube S, which is then closed with a cork. The second side-tube is then closed with a calcium chloride tube, and the condenser, which is completely dry, is so inserted that it does not touch the wall of the tube at any point. The micro-burner is placed exactly under the centre of the boiling-flask at a distance of 1–3 cm. below the gauze, so that the luminous point of the flame just touches the latter. If the heating is too strong it will be found that the liquid merely boils in the descending tube and that the nozzle does not act ; under these conditions the thermometer reading is not constant. This difficulty is overcome by reducing the flame and raising it to a shorter distance from the gauze.

When the temperature has remained constant to within 0·002° C. throughout five minutes, the first tablet, weighing 15–25 mgm., is added on through the side-tube S. It is sufficient to weigh this to within 0·1 mgm. After two to three minutes the second temperature reading is taken. If this remains constant throughout a further two minutes, a second tablet is added, and the third reading of the temperature is taken after this has remained constant for another two to three minutes.

Sometimes, particularly when using pyridine or glacial acetic acid, the thermometer bulb is bombarded by liquid which foams and spurts into the descending tube ; this can be avoided by raising the thermometer to such a point that its bulb is situated 1–2 cm. only below the nozzle.

For substances and solvents which are liable to be superheated, A. Rieche [1] recommends boiling the solvent in a paraffin bath.

In a new model [2] the baffle-cone is replaced by a syphon trap, which has the form of a small very high beaker. Through this

[1] *Chem. Ztg.*, **52**, 923 (1928).
[2] A. Rieche : *Mikrochem.*, **12**, 129 (1933).

neither hot spray from the boiling-vessel nor cold drops from the condenser can come in contact with the thermometer.[1] About one-third of the boiling-vessel is immersed in the paraffin bath, which is kept about 25°–40° C. above the boiling-point of the solvent by means of a micro-burner. Constant boiling only appears after twenty-five to thirty minutes. The apparatus is protected from air currents by wrapping in cellulose wadding. The nozzle should blow the solvent on to the bulb of the thermometer in blasts which follow one another rapidly.

According to the experience of the author, the first values are better than those after adding the second tablet.

Molecular Weight Determination in a Melting-point Apparatus, according to K. Rast [2]

Basis of the Method

The observation by M. Jouniaux,[3] that, in addition to its value as a solvent for many substances, camphor produces a high molecular depression of the melting-point, has been used by K. Rast for the development of an excellent method for the determination of molecular weight. Whilst the melting-point depression of 1 molecule of dissolved substance per kilogram of solvent is only about 1·86° C. in water, it attains the value of 40° C. when camphor is used. On this basis, Rast produced a method which exceeds all others in simplicity and in the smallness of the quantities which are required. This method can naturally not be used for those substances which decompose below the melting-point of camphor, *e.g.*, many carotinoids, alkannin, etc. In order to make such substances available for Rast's method, J. Pirsch [4] has proposed different hydro-aromatic substances of lower melting-point and large molecular depression of the melting-point as solvents. The new solvents now make it possible for us to check the molecular weight obtained by the camphor method by means of other solvents.

Every substance which is considered as a solvent is carefully tested to see that it satisfies these conditions :—

1. The production of a high molecular depression of the melting-point.

2. Good solvent power.

3. The molecular melting-point depression must be independent of the concentration of the dissolved substance.

[1] The apparatus is manufactured by Siebert and Kühn, G. m. b. h., Kassel.
[2] *Ber. dtsch. chem. Ges.*, **55**, 1051 and 3727 (1922).
[3] *Bull. soc. chim. France*, **11**, 722, 993 (1912); *Compt. Rend. Acad. Sci. Paris*, **154**, 1592 (1912).
[4] *Ber. dtsch. chem. Ges.*, **65**, 862, 865, 1227, 1839 (1932); **66**, 349, 506, 815, 1694 (1933); **67**, 101, 1115, 1303 (1934); **68**, 67 (1935).

Further, it is obvious that an accurate determination is possible only if the substance dissolves to a clear solution and does not react with the solvent. The sharper the melting-point, the more accurate is the analysis.

The determination of the molecular melting-point depression is described on p. 240. It may here be pointed out that this simple method for the above determination also enables us to calculate the molecular heat of fusion of the solvent according to the Van't Hoff equation :—

$$W = \frac{RT^2 \cdot M}{100 \cdot K},$$

where W = molecular heat of fusion, R = gas constant, T = absolute temperature, M = molecular weight of the test substance and K = molecular lowering of melting-point.

Requirements

One requires an ordinary melting-point apparatus—a micro-Kjeldahl flask, which is filled to the neck with concentrated sulphuric acid, is best [1]; also, four thermometers, each of 50° C. range, to form a set from 0°–200° C. These are graduated in fifths of a degree. For determinations in camphor a thermometer of 120°–180° C., manufactured according to F. Pregl's specification by the firm of P. Haack, may be used.

Melting-point capillaries are conically drawn out from cleaned test-tubes in the blow-pipe flame or in a Bunsen burner. These should be about 4 cm. long and the narrow end, which is sealed, should have a bore of 2·5 mm., and the wider end a bore of at least 3 mm. (Fig. 63). Care should be taken, when sealing the narrow end, to produce a nicely rounded hemisphere without forming a point or a heavy drop of glass. It is advisable to store such capillaries in a considerable quantity, and, more particularly, to store in quantity the auxiliary capillaries and glass rods used for introducing the substance in the manner now about to be described. For this purpose, there are required ordinary melting-point capillaries of $\frac{1}{2}$ mm., or at most, 1 mm. bore, and a length of at least 5 cm., and glass rods 6 cm. long which can easily be passed through the bore of these capillaries. For introducing the solvent, capillaries are required of a bore of 1–1$\frac{1}{2}$ mm., 5 cm. long and provided with glass rods of a length of at least 6 cm., which are easily passed through these. The capillaries for introducing liquids of high and low boiling-points are described later (pp. 2401–2421).

The melting-point is read by a lens.

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[1] For the determination of melting-points with solvents, the melting-points of which lie below 80° C., water may replace the sulphuric acid.

Solvents

1. *Camphor.* A.R. Ten to twenty grams are ground to a uniform powder in a mortar whilst sprinkling with a little ether. By spreading it in a thin layer on filter paper and protecting it from dust by covering with the paper for some time, the whole of the ether is evaporated. The camphor for the analysis is kept in a wide-necked bottle with ground-in stopper. A good preparation possesses a melting-point of 176°–180° C. and causes a molecular melting-point depression of 38°–40° C. Solvents suggested by J. Pirsch [1] are :—

Camphene. [2] M.p., 49° C. Molecular lowering of melting-point, K [3] = 31° C. According to a published statement, this has often been found very satisfactory, owing to its good solvent power and high molecular melting-point depression.

Bornylamine. [4] M.p., 164° C. K = 40·6° C. In consequence of its basic character it is particularly suitable as a solvent for alkaloids and basic substances in general.

Camphoquinone. [5] M.p., 190° C. K = 45·7° C. This may be used instead of camphor as a solvent for substances of high melting-point.

· *Camphenilone.* [6] M.p., 38° C. K = 64° C.

Dihydro-α-dicyclopentadiene-one-3. [7] Owing to its particularly large molecular depression of freezing-point, its use makes it possible to determine the molecular weight of substances, the molecular weight of which is proportionately high.

Cyclo-pentadecanone [8] (" Exalton "). M.p., 65·6° C. K = 21·3°C. This is a good solvent for azo dyes, many quinones and carotinoids, and especially for sterols and their derivatives. Exalton of a high degree of purity can be obtained from Schering-Kahlbaum, and may be used without further purification for this determination.

Besides these substances, numerous other solvents are used for this work. A. Zinke's [9] perylene is used as solvent for anthraquinones of high melting-point and perylene derivatives. K = 25·7° C. Pastak's [10] 2, 4, 6-trinitrotoluene is used for polynitro compounds ; K = 11·5° C.

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[1] Other solvents proposed by J. Pirsch are to be found in the articles referred to in footnotes 5 to 7.
[2] On the preparation and purification, see *Ber. dtsch. chem. Ges.*, **65**, 862 (1932).
[3] K = Molecular depression of melting-point.
[4] Preparation and purification, *Ber. dtsch. chem. Ges.*, **65**, 1227 (1932).
[5] Preparation and purification, *Ber. dtsch. chem. Ges.*, **66**, 349 (1933).
[6] Preparation and purification, *Ber. dtsch. chem. Ges.*, **66**, 1694 (1933).
[7] Preparation and purification, *Ber. dtsch. chem. Ges.*, **67**, 1115 (1934).
[8] *An. Soc. espan. Fis. Quim.*, **33**, 438 (1935).
[9] *Ber. dtsch. chem. Ges.*, **58**, 2388 (1925).
[10] *Bull. Soc. chim. France* [4] **39**, 82 (1926).

Determination of the Melting-point and the Molecular Lowering of the Melting-point of the Solvent

Before using a new solvent, its melting-point and the molecular lowering of its melting-point must first be obtained most accurately. The former is determined as previously described, taking at least three readings, which are accurate to $\pm$ 0·1° C.

To find the molecular lowering of the melting-point, the melting point of an analytically pure substance of known molecular weight— *e.g.*, naphthalene or benzoic acid—is determined as on p. 243, after adding ten times its weight of solvent. Several readings accurate to $\pm$ 0·1° C. are taken. The molecular melting-point depression K of the solvent is calculated from the equation :—

$$K = \frac{M \cdot L \cdot \Delta_t}{1000 \cdot S}, \text{ where}$$

M = molecular weight of the substance added.
L = weight of solvent.
Δ_t = difference between the melting-points of the solvent and solution.
S = weight of substance added.

The Course of the Determination

Weighing of Solids According to F. Pregl's Method. The carefully cleaned melting-point capillary is placed with the opening uppermost in a small empty counterpoised flask (p. 13) with a not too narrow neck ; this is placed on the balance pan by means of a forceps and the weight determined accurately to 0·001 mgm. after a few minutes. To avoid the application of the 10 mgm. weight, one counterpoises the flask with the melting point capillary so that the rider will be placed on the beam to read less than 5 mgm. After weighing, the flask and capillary, which must not be touched again by the hand, are placed on a notebook in front of the balance. A small amount—about 0·2–0·3 mgm.—of material is placed on a clean watch-glass and carefully pressed up into the narrow auxiliary capillary, which is then carefully brushed with a marten-hair brush and introduced to the bottom of the melting-point capillary and its contents expelled by the use of the corresponding glass rod, whilst the auxiliary capillary is raised about 2–4 mm. Very hard, granulated materials, which cannot be pressed in, are pushed with the spatula into the auxiliary capillary, which is in a slanting position and transferred from this to the melting-point capillary by correspondingly tilting the counterpoised flask. After the weight of substance has now been determined accurately, the solvent is added in the same way with the help of the wider auxiliary capillary,

only taking care not to push this capillary up to the bottom of the melting-point capillary, because in that case particles of the material already weighed might stick to its edge. In this case it is advisable to fill the solvent into the melting-point capillary to a height of 4–6 mm., by pushing it out of the auxiliary capillary, as described, with the glass rod. Sometimes the weight shows that a further charge of the solvent is necessary in order to obtain approximately ten times the proportion of solvent to the substance under investigation. The third weighing then follows, from which the exact weight of solvent is obtained. The middle of the conical capillary is then sealed by heating over a small flame and drawn out to a thread which is not too thin and about 4 cm. long. The hollow portion, with the weighed charge, should be about 15–20 mm. long (Fig. 63).
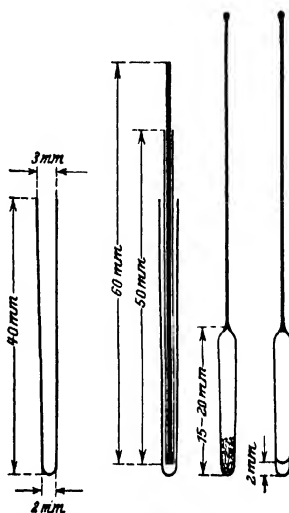
FIG. 63.—*I.* Capillary for molecular weight determination by Rast's method. *II.* Charging the same. *III.* After sealing. *IV.* After melting and resolidification of the contents.

Oily and Viscous Substances. By J. Pirsch's method, a glass rod about 0·7 mm. in diameter is sealed exactly in the centre of a small glass tube which is 80–90 mm. long, open at both ends, and of, at most, 2·5 mm. external diameter (Fig. 64). The glass rod projects 1–1·5 mm. on the one side. By carefully dipping the glass rod sideways into the material to be used, as much of this adheres as is necessary for one weighing. This guide tube is held with the
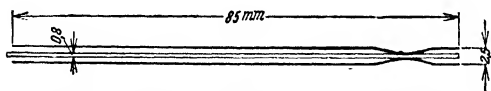
FIG. 64.—Small guide tube for oily and viscous liquids.

thumb and first finger of the right hand for the introduction of the substance into the weighed melting-point tube and the substance is transferred to the bottom of the melting-point tube, which is held vertically. The protecting tube makes it impossible to moisten the side of the melting-point tube. The solvent is then weighed just as with solid substances.

Liquids of High Boiling-point. By A. Soltys' method, the

adherence of even the slightest traces of liquid to the side of the weighed melting-point capillary is avoided. The liquid to be weighed is introduced by means of a glass tube, the end of which is drawn out to a hair capillary, 1·5-2 mm. long. Such filling tubes are most simply produced by drawing out an ordinary glass tube of suitable dimensions, as it is difficult to prepare them from thin-walled capillaries. The hair capillary is filled by dipping it into the liquid concerned, wiped externally, introduced to the bottom of the melting-point capillary in such a manner that the point does not touch the inner wall, and the contents, which should weigh 0·2-0·3 mgm., are blown out on to the floor of the capillary (Fig. 65). The second weighing should be carried out quickly, after which the requisite amount of solvent is introduced and the determination continued as already described for solids ; one should not mix the substance by rotation in a preliminary bath, as otherwise traces of liquid may easily adhere to the upper end of the scaled capillary. Owing to the fact that the solvent absorbs the liquid, admixture is so
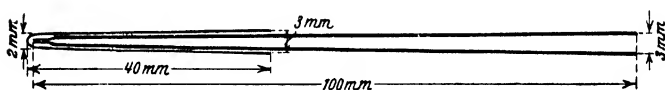
Fig. 65.—Introducing the liquid into the melting-point capillary.

good that the melting-point becomes constant after the first melting and setting in the melting-point apparatus.

Liquids of Low Boiling-point. By J. Pirsch's [1] method the solvent is first weighed into the melting-point capillary of 70 mm. length as on p. 240. To this, the liquid weighed in a fine capillary is added before scaling. The capillary for the liquid consists of a capillary sealed on one side, of about 1 mm. bore, which is drawn out to a hair capillary 10 mm. long, at a distance of 8-9 mm. from the scaled end. To introduce the liquid, the fine tip of the weighed capillary is immersed in a small high dish, in which the height of the liquid is a few millimetres. The wide end of the capillary must project at least 6 mm. above the dish. The capillary is lifted out of the liquid by means of a warm forceps for a few seconds and then replaced, so that the liquid rises in it on cooling. After sufficient liquid has been sucked up to fill the conical upper part, the capillary is lifted out of the liquid by means of a bone forceps, whereby air is sucked back through the hair capillary. The extremely narrow and proportionately high air column thus formed in the hair capillary completely prevents leakage of vapour from the liquid. The capillary is now weighed on the balance pan.

[1] *Ber. dtsch. chem. Ges.*, **65**, 865 (1932).

It is then allowed to fall into the 70 mm. long melting-point capillary, in which the solvent has been weighed as already stated. Any conduction of heat to the capillary through the longer melting-point capillary is now prevented by quickly sealing. If, however, in spite of this, liquid should enter the capillary, this causes no error, as experience has shown, because the vapourised liquid cannot diffuse into the melting-point capillary sufficiently quickly.

The mixing together of the liquids is undertaken either through repeatedly immersing the prepared capillary in a bath warmed to the melting-point of the solvent or by carefully and repeatedly warming the wide part of the sealed-in capillary and solvent.

Reading of the Melting-point

Before carrying out the reading one has to mix the substance and solvent thoroughly together. For this purpose a so-called mixing bath is prepared, either with water or with sulphuric acid according to the solvent to be used, which is warmed to $1°-2°$ C. above the melting-point of the solvent.

The capillary is then held by the glass thread, the whole of the hollow part is immersed in the bath, and the substance is completely dissolved by energetically rotating under observation with the lens. Should the substance not be completely dissolved even after rotating for a long time, a melting-point determination is useless. If the substance goes into solution except for a very small residue, it will probably be dissolved in a fresh weighing using a larger amount of solvent. If it remains undissolved, another solvent must be examined.

After cooling, the glass thread of the capillary is attached, by means of a fine rubber ring, to the corresponding short-range thermometer, and this is introduced into the melting-point apparatus in the usual way. It is best to heat the melting-point apparatus with the pilot flame of a Bunsen burner, and to regulate the warming of the bath so that the rise in temperature is $2°$ C. per minute at most. In the neighbourhood of the melting-point the heating is carried out more slowly. A few degrees below the melting-points the contents of the capillary are converted to a turbid liquid in which a crystalline network can be observed with the lens. The network originally occupies the whole of the liquid, but on raising the temperature the crystals vanish from the top, and the last of them, which lie at the bottom, usually disappear with a slight eddying motion. At this point the temperature is read accurately to $0·5°$ C. The whole is then cooled, and a second reading taken to check the first.

16—2

Calculation

$$M = \frac{1000 . K . S}{L . \varDelta_t}.$$

K = molecular depression of freezing-point of solvent, as determined on p. 240.

S = weight of substance.

L = weight of solvent.

$\varDelta_t$ = depression of melting-point.

Example :—

Camphor as Solvent.

Nitrobenzene, $C_6H_5NO_2$.   *Mol. wt.*, 123·04.

$$L = \quad 4·599 \text{ mgm.}$$
$$K = \quad 38° \text{ C.}$$
$$S = \quad 0·237 \text{ mgm.}$$
$$\varDelta_t = \quad 15·7° \text{ C.}$$
$$M = 125$$

Carbon Disulphide, CS_2.   *Mol. wt.*, 76·1.

$$L = 14·549 \text{ mgm.}$$
$$K = 31·08° \text{ C.}$$
$$S = \quad 0·807 \text{ mgm.}$$
$$\varDelta_t = 22·0° \text{ C.}$$
$$M = 78·3$$

Determination of Molecular Weight according to the Osmotic Method of G. Barger [1]

Basis of the Method

Incidentally to an investigation on mushrooms, Professor L. Frerrera observed variations of the vapour pressure in salt solutions, and begged G. Barger, because he saw a molecular weight determination in it, to initiate investigations concerning this.

G. Barger's method is based on the following principle : If solutions of different concentrations are placed in a closed system, solvent passes from the solution which is osmotically weaker to that which is osmotically stronger by isothermal distillation until the same osmotic pressure is set up in both. If a solution of known concentration of the substance to be examined is prepared and tested against comparison solutions of known molecular concentration, the molecular weight may be very accurately determined. For the determination, small drops of the solutions of the substance and of the comparison solution are placed alternately between small

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[1] *J. Chem. Soc.*, **85**, 286 (1904) and *Ber. dtsch. chem. Ges.*, **37**, 1754 (1904).

air bubbles. The alteration of the distance between the two meniscuses of a drop, owing to isothermal distillation, is determined under the microscope by means of an ocular micrometer. If one of the comparison solutions shows no osmotic alteration, it is used for the calculation of the molecular weight of the substance, as given on p. 284.

No special experimental demands are made in regard to the carrying out of this process, and it possesses a wide range of application owing to the different solvents and mixtures of solvents which may be used. Also, solvents which are not analytically pure may be used for it. Through the simple devices for apparatus it can be used for the determination of molecular weight in every laboratory ; the only drawback is that rather a long time elapses before osmotic equilibrium is reached. The time depends on the vapour pressure of the solvent, e.g., acetone, about twelve hours ; pyridine or water, a few days.

Devices for ˙Apparatus and Course of Analysis

The measuring capillary is best prepared from an ordinary glass tube with walls 2 mm. thick and of about 15 mm. external diameter. The carefully cleaned tube is drawn out to a bore of 0·9–1·2 mm. for organic solvents and 1·5–2 mm. for water, and cut in pieces of about 150 mm. The capillaries must be accurately checked before filling ; above all, they must show the same inner diameter.

Any microscope which with a focal length of about 18 mm. shows a magnification of sixty to one hundredfold. An ocular micrometer is inserted in the eyepiece.

The slides with the capillaries are kept in Petri dishes.

On account of its excellent solvent power, pyridine is the most suitable solvent ; a mixture of pyridine with acetone, and solvents of low boiling-point such as ether, acetone, glacial acetic acid and alcohol are also used.

Azobenzene, cane sugar, benzene and urea are used as comparison substances.

The comparison solutions are prepared [1] after the suitable solvent has been found by a preliminary test. Azobenzene is best because it makes the solution clearly visible. Much time and trouble are wasted if the comparison solutions are decanted into bulbs after their preparation. It is better to keep those prepared from non-volatile solvents in small flasks with ground-in stoppers. With volatile solvents, the solutions should be freshly prepared ; for this an M/10 comparison solution is first prepared and then a series of

[1] E. Berl and O. Hefter : *Ann*, **478**, 235 (1930).

solutions by dilution with the solvent from a micro-burette (see table below.

Because the concentration of the solution of the substance to be examined must be known in order to calculate the molecular weight required, if sufficient material is at one's disposal as much as will yield a 0·1–0·3 per cent. solution is weighed into a graduated flask of 1–3 c.c. capacity. If the material is insufficient for this, it is preferable to determine the percentage by weighing the material and the solvent. To calculate the concentration of solution from this, the determination of the specific gravity is necessary. This is found exactly as in the determination of the rotatory power

TABLE OF AN AZOBENZENE-ACETONE SERIES OF DILUTIONS
ACCORDING TO E. BERL AND O. HEFTER [1]

Hypothetical Molecular Weight.	gm. of Azobenzene in 10 c.c. of Acetone.	Normality.	Dilution.	
			c.c. Acetone.	c.c. M/10 Azobenzene Solution.
100	0·18212	0·1		
120	0·15175	0·0835	40	8·0
150	0·12140	0·0667	30	15·0
170	0·10712	0·0588	30	21·0
200	0·09105	0·0500	25	25·0
220	0·082773	0·0455	20	24·0
250	0·072840	0·0400	20	30·0
270	0·067445	0·0370	15	25·5
300	0·06070	0·0334	15	30·0
320	0·056906	0·0313	10	22·0
350	0 052029	0·0286	10	25·0
370	0·049217	0·0270	10	27·0
400	0·045525	0·0250	10	30·0

(p. 251). Similarly, the polarisation solutions may also be used for the determination of the molecular weight.

The filling of the capillaries requires some practice, but is, however, by no means difficult and only takes a short time. The measuring capillary is held between the thumb and middle finger, and whilst one end is covered with the first finger the other is dipped in the comparison solution; thus only a small amount of solution enters the capillary. This is then raised, turned into a horizontal position, the first finger removed, and, by tilting, the droplet is moved about 3 mm. into the capillary. Similarly, one droplet of the solution of the substance is transferred to the capillary. This is repeated till about 7 droplets, of the two solutions alternately, are in the capillary; they are allowed to enter it to an extent such that the last is about 1 cm. from the opening of the capillary.

[1] Further tables will be found in *Ann.*, **478**, 235 (1930).

Finally, with the capillary horizontal, both ends are sealed with a micro-burner. Aqueous solutions are occasionally difficult to slide into the capillary. The end of the capillary which is farthest away from the solution may therefore be gently warmed and then covered with the finger, the drop is therefore sucked in on cooling. If very volatile solvents (ether, carbon disulphide) are used, the capillary is best sealed with wax or paraffin. The other measuring capillaries are filled with comparison solutions of the series in diminishing concentration.

The prepared measuring capillaries are pasted, at both ends, on slides by means of wax or narrow leucoplastic strips ; the slides are then labelled (Fig. 66).

The reading is only taken for drops *2* to *6*, because both the
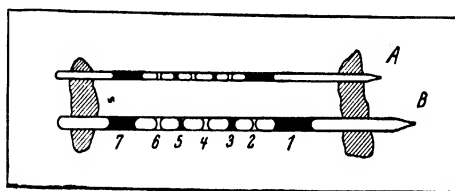
Fig. 66.

outside drops undergo abnormal changes through evaporation into the adjacent larger volume of air.

For the measurement the slide with capillary is placed in a rectangular Petri dish, and water at room temperature added until the capillary is just covered ; it is thus protected from room temperature on the one hand while the image is made clearer on the other.

The microscope is focussed exactly on the axis of the capillary, because in this position both meniscuses of a drop are most sharply defined. The smallest interval between the meniscuses in the axis of the capillary is measured by exactly covering one meniscus with the zero of the micrometer through moving the Petri dish, and the other is read accurately to 2–3μ. Thus, in Fig. 66, drops *2* to *6* are measured and the nearest comparison solution is then measured. The interval before the next reading depends on the vapour pressure of the solvent used—with ether, a few minutes; alcohol, an hour ; pyridine and water, about two days. If the solutions are very dilute the time is correspondingly larger.

The readings which now follow must be taken at the same temperature. By this, one finds whether the distance between the meniscuses of a drop have become either larger or smaller. From the series of compression solutions, only those are used for the

determination, of which the one just permits an increase and the other a decrease in the distance between the meniscuses to be recognised, because only in the most exceptional cases is no change to be observed. Thus the smallest isothermal distillation has occurred between the droplets of the solution of the substance, of known concentration, and that of the comparison solution, of similar known molecular concentration.

Example. Molecular weight determinations of grape-sugar (dextrose) against an aqueous solution of cane sugar (molecular

Cane Sugar.	Time in Hours.	II.	III.	IV.	V.	VI.	Sum.
0·05 M. . .	18	+230	−97	+71	−79	+71	+548
0·10 M . .	18	+ 26	−18	+25	−31	+30	+130
0·12 M. . .	21	+ 6	− 4	+ 9	− 4	+ 4	+ 27
0·13 M. . .	22	+ 8	+ 3	+ 5	− 1	+ 5	+ 16
0·14 M. . .	22	− 1	0	− 2	+ 2	− 2	− 7
0·15 M. . .	18	− 3	+ 8	0	+ 9	− 4	− 24
0·20 M. . .	18	− 41	+55	−57	+53	−45	−251
0·25 M. . .	18	− 75	+85	−81	+65	−78	−384

weight, 342). Concentration of the grape-sugar solution = 2·502 per cent.

Only the alteration of the meniscus as relative to the first reading, after the time in column 2, is given, not the actual variation of the droplets. From the five droplets measured, II., IV., VI. are grape-sugar solution and III. and V. are cane-sugar solution. The last column quotes the sum of the changes in the five droplets.

From the example one sees that the osmotic pressure of the 2·502 per cent. grape-sugar solution corresponds to one between 0·13 and 0·14 molecular solution. From this, one calculates the molecular weights $\dfrac{2\cdot502 \cdot 10}{0\cdot13} = 179$ and $\dfrac{2\cdot502 \times 10}{0\cdot14} = 192$.

Molecular weight calculated from formula, $C_6H_{12}O_6$, = 180·09.

Calculation

$$\text{Molecular weight} = \frac{\text{Concentration of solution of substance} \times 10}{\text{Molecular concentration of the comparison solution}}$$

An apparent source of error may here be mentioned. On filling, the droplets pass through the part of the capillary which was moistened by the previous droplets, so that mixture occurs. The observed change is therefore somewhat lower than if the droplets had been introduced without moistening by the other solutions. However, because we only wish to know which solutions have the

same osmotic pressures and do not wish to measure the size of the difference, this only makes the method somewhat less sensitive.

K. Rast [1] has modified the method so that only one drop of the solution of the material and one of the comparison solution is introduced into the capillary. The alterations for both solutions are measured against a line placed on the slide between two droplets.

Of further methods based on the same principle, the author has no personal knowledge ; reference should therefore be made to the originals :—

K. Schwarz : *Monatshefte*, **53/54**, 926 (1929).

E. Berl and O. Hefter : *Ann.*, **478**, 235 (1930).

R. Singer : *Ann.*, **478**, 246 (1930).

I. K. Spiers : *J. Amer. Chem. Soc.*, **55**, 250 (1922).

IV. Determination of Rotatory Power by E. Fischer's Method [2]

The first attempts to determine the optical rotatory power of small quantities of substances were carried out in the laboratory of F. Emich, by J. Donau,[3] with capillaries of 0·5 mm. diameter. E. Fischer developed the process in a form which enabled it to be generally applied.

Apparatus

Small weighing bottles of 1·5 c.c. capacity, with ground-in stoppers (Fig. 67*b*).

Charging pyknometers, according to F. Pregl's specification, of about 0·12 and 0·22 c.c. capacity [4] (Fig. 67*a*).

E. Fischer's polarisation tubes :—[5]

$$\begin{array}{llll} \text{1·6 mm. diameter} & \text{(5 cm. long,} & \text{0·1 c.c. capacity)} \\ \text{1·6 mm.} \quad ,, & \text{(10 cm.} \quad ,, & \text{0·2 c.c.} \quad ,, \quad) \\ \text{2·5 mm.} \quad ,, & \text{(10 cm.} \quad ,, & \text{0·5 c.c.} \quad ,, \quad) \end{array}$$

The polarisation apparatus (Schmidt and Haensch, Berlin, p. 42) is a half-shadow apparatus, with a bi- or tripartite field of view. Monochromatic light is obtained through a monochromator which spectroscopically analyses the white light from a Nernst lamp, or,

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[1] *Ber. dtsch. chem. Ges.*, **54**, 1979 (1921).
[2] *Ber. dtsch. chem. Ges.*, **44**, 129 (1911).
[3] *Monatshefte*, **29**, 333 (1908).
[4] Pregl's original pyknometer had a capacity of about 0·9 c.c.
[5] H. Naumann, *Biochem. Z.*, **211**, 239 (1929), prepared the tubes from black glass and with frosted walls, by which means internal reflections, which previously interfered with the readings, were avoided. These tubes can be obtained from the firm of Schmidt and Haensch, Berlin.

preferably, from an electric incandescent lamp. With this, measurements with different wave-lengths (determinations of rotatory dispersion) are possible. For extremely accurate measurements with yellow light (D-line, 589 mμ), the sodium-gas-filled lamp of Osram, Berlin, mounted by C. Zeiss, Jena, instead of the expensive monochromator, has been greatly approved ; for red light, the Siemens' cadmium lamp, with or without filter, which radiates principally the line 643 mμ.

Yet another series of gas-filled lamps, which may all be used in the same socket, has been constructed ; these lamps admit of measurements with monochromatic light in almost every part of the spectrum and are extremely convenient to handle, so that it should only be necessary to fall back upon the monochromator in conjunction with a corresponding source of light in exceptional cases, when extreme range of the spectrum or particularly high strength of light is necessary.

Course of the Determination

Solutions for polarisation may be prepared in two ways.

(a) By dissolving a weighed amount of substance in a calibrated measuring flask and obtaining the concentration c in gm./100 c.c.

a.   b.   c.
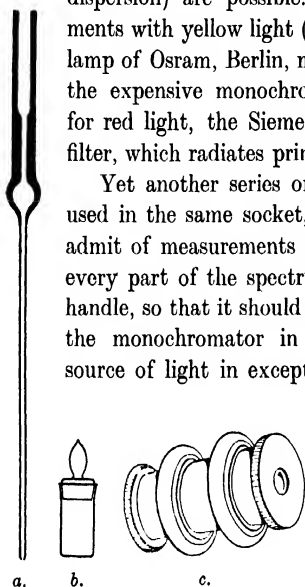
Fig. 67.—a, Charging pyknometer of the firm of P. Haack, Vienna. (Actual size.)   b, Small weighing bottle. c, Polarisation tube.

This is best if one has sufficient material. If, however, only a few milligrams are available, the second method is recommended.

(b) The very carefully cleaned weighing bottle (Fig. 67b) is placed on the balance and weighed after ten minutes. It is then transferred, by means of a forceps or a chamois leather, to the notebook in front of the balance, 3–10 mgm. are carefully placed in it with a spatula, the stopper loosely inserted, and the bottle weighed again after five minutes.

The solvent is allowed to flow from a fine scale-pipette carefully down the wall of the weighing bottle on to the substance. About 0·15 c.c. or 0·25 c.c. are added, according to the tubes and pyknometers selected. Then the ground-in stopper is fitted in tightly and after five minutes the solvent is weighed. If the substance. has not dissolved completely it is carefully shaken around.

From the two weights the percentage content of the solution (gm. substance/100 gm. solution) is found ; to ascertain the concentration c, determination of the density (d^t_4) is necessary.

Determination of the Density

The charging pyknometer (Fig. 67a) is washed with water, alcohol, and ether, dried by means of the pump, cleaned with a chamois leather and placed beside the balance. Because all weighings must be carried out at exactly the same temperature, a few cubic centimetres of distilled water and the solution are placed close together in the balance case. After ten minutes the empty pyknometer is placed on the balance by means of a fork and weighed accurately to 0·01 mgm. after five minutes more. The pyknometer should not be touched again by hand after this, but is removed with the fork and held in chamois leather whilst pressure tubing, with mouthpiece, is put on it. The tip is then immersed in the water at room temperature and this is carefully sucked through, to not more than 1–2 mm. above the mark ; the pyknometer is immediately turned into a horizontal position and the tubing removed without compressing it. Whilst the pyknometer is held horizontal, the water is removed from the outer wall of the tip with filter paper, without touching the outlet capillary with this. The pyknometer is then tilted, with the tip lowest, through 30–40 degrees, and water is absorbed from the tip with filter paper, which has been moistened slightly, till the water stands exactly at the mark.

If, on filling the pyknometer, the water is drawn more than 1 mm. above the mark, then, to enable the water adhering to the wall of the capillary to flow off, the pyknometer is kept tilted for two minutes, after which water is again sucked up to the mark. After some unsuccessful experiments no difficulty is experienced in sucking the water not more than 1 mm. past the mark. If the water is drawn several millimetres above the mark, the accuracy of the determination is affected so much that the drying and filling of the pyknometer must be repeated.

In spite of the rather long manipulation, with which a slight absorption of heat is inevitable, the weighing is carried out after five minutes. Finally, the polarisation solution is weighed with the same pyknometer and at exactly the same temperature.

Filling the Polarisation Tube

After the observation tube has been cleaned with water, alcohol, and ether and dried by the pump, a small glass plate is put on it and the stopper is screwed up. One must be careful to avoid air bubbles on pouring in the solution. For this, the pyknometer is removed from the balance and held by its upper end, whilst the tip passes to the bottom of the small tube. When the tip touches the small glass plate, the solution is allowed to flow in and the pyknometer is

raised accordingly. When the solution appears above the edge of the tube, the pyknometer is removed, the second small plate of glass put on, and the cover immediately screwed up. The small cover-plate should not be screwed too tightly, because in that case the double refraction phenomena of the glass may occur, which might injure the uniformity of the field of view and lead to a zero displacement. For this reason, all measurements are to be repeated after turning the tube round.

After looking through the tube to ascertain that air bubbles are absent, one places it in the polarisation apparatus. Streaks, which blur the field of view, are due to differences of temperature and disappear spontaneously after some time through temperature equalisation. By moving the observation tube in the groove of the polarimeter, the best position for sharp reading is discovered. The mean of six to eight readings, from dark to clear and *vice versâ*, is taken. With some practice, an accuracy of $\pm$ 10° C. is easily attained.

The zero of the apparatus at the time is determined by filling the tube with the solvent used, at the temperature of the measurement.

Calculation

The specific rotatory power $[\alpha]$ is the angle of rotation α of the plane of polarised light by a solution of 1 gm. of material in 1 c.c. when the beam of light passes through a layer 1 dm. long.

If the concentration c (gm./100 c.c.) has been determined with the measuring flask, then

$$[\alpha]_D^t = \frac{\alpha \cdot 100}{l \cdot c} \tag{1}$$

where t = temperature of observation, D = wavelength of the light, α = angle measured in degrees and l = length of the tube in decimetres.

If the pyknometer has been used, d_4^t, the density of the solution referred to water at 4° C., must first be ascertained. For this, W, the weight in grams of water in the pyknometer at $t°$ C., is divided by the density of the water at this temperature,[1] giving W_4, the volume of the pyknometer in cubic centimetres. The reduced density is :—

$$d_4^t = \frac{L^t}{W_4} \tag{2}$$

where L^t is the weight in grams of the solution in the pyknometer at the temperature t. For the specific rotation, then :—

$$[\alpha]_D^t = \frac{\alpha \cdot L}{l \cdot S \cdot d_4^t}. \tag{3}$$

[1] Tables : T. W. Küster : "Logarithmische Richentafel," Editions 35–40 ; p. 186 ; more fully in the "Chemiker Kalender," 1930, i., p. 43.

α = angle of rotation read.

L = weight of solution in weighing bottle.

l = length of tube in decimetres.

S = weight of substance.

Example :—

1. Cane sugar.

28·025 mgm. in 5 c.c. water.

According to (1) :—

$$c = 5{\cdot}605 ; \quad l = 0{\cdot}5 \text{ dm.} ; \quad \alpha_D^{20} = +\ 1{\cdot}87.$$

$$[\alpha]_D^{20} = \left(\frac{+\ 1{\cdot}87 \times 100}{5{\cdot}605 \times 0{\cdot}5} \right) = +\ 66{\cdot}7^\circ.$$

2. Cane sugar.

In the weighing-bottle, $S = 8{\cdot}920$ mgm. ; $L = 339{\cdot}62$ mgm.

In the pyknometer, $W = 0{\cdot}2211$ gm. at 20° C ; $W_4 = 0{\cdot}2235$ c.c. ; $L^t = 0{\cdot}22541$ gm. at 20° C.

From (2) and (3) :—

$$d_4^{20} = \frac{0{\cdot}22541}{0{\cdot}22350} = 1{\cdot}0085 \text{ gm./c.c.}$$

$$[\alpha]_D^{20} = +\ \frac{0{\cdot}88^\circ \times 339{\cdot}62}{0{\cdot}5 \times 8{\cdot}920 \times 1{\cdot}0085} = +\ 66{\cdot}4^\circ.$$

V. Determination of Molecular Refraction

Refractive Index

For the determination of the refractive index on small amounts of liquid or solution, the Abbé refractometer is particularly suitable. Only 1 drop is necessary and the refractive index can be read accurately to $\pm$ 0·0001.

The Abbé refractometer is based on the principle of the determination of the limiting angle of total reflection in a thin layer of liquid between two prisms of greater refractive index. Readings from $n_D = 1{\cdot}3000$ to $n_D = 1{\cdot}7000$ can be taken. For illumination, daylight can be used as well as an electric bulb. The apparatus possesses a colour compensator, which consists of two direct vision Amici prisms for the sodium line. The refracting surfaces of the compensator, which are in a plane perpendicular to the optical axis of the telescope, can be turned into various angles with each other. This arrangement acts optically as a single prism with continuously variable dispersion. If the compensator is turned so that the boundary line between light and dark which falls in the middle of the cross wires appears quite colourless, the refractive index for the sodium line n_D can be read directly on the scale in spite of illumination with white light.

To carry out the determination, the instrument is opened and tilted so that the exposed surface of the fixed prism is horizontal. A drop of liquid is then placed on this surface with a pipette or with a rounded glass rod, the movable prism is clapped on it, and the apparatus fastened and then turned up again. The optical instrument is illuminated by the mirror and the screw of the scale supplied is turned till the line of separation between light and dark appears in the field. The colour compensator is now adjusted so that this line is quite colourless, it is then brought exactly on the centre of the cross wires and n_{υ} is read on the scale with the lens. The fourth decimal place is estimated.

The refractometer is made so that water from a thermostat can be run through both prisms. In practice, it is usually sufficient to place the refractometer, provided with a screwed-in thermometer, in the balance room, in which the density of the liquid is determined. The molecular refraction is, indeed, independent of the temperature, but care must be taken that the density and refractive index are determined at exactly the same temperature. The apparatus is calibrated with a drop of water, for which $n_{\upsilon} = 1\cdot3330$ at $20°$ C.

Density

The absolute specific gravity of the liquid, referred to water at $4°$ C., is determined with the precision weighing-pipette shown in Fig. 68; the determination is made by the micropolarisation method (p. 251).

Calculation

The molecular refraction is calculated according to the equation :

$$MR = \frac{M}{d}\left(\frac{n^2 - 1}{n^2 + 2}\right),$$

where M = molecular weight of the substances. In the " Refraktometrisches Hilfsbuch " [1] tables are given in which $\dfrac{n^2 - 1}{n^2 + 2}$ may be read for values of n from $1\cdot3000$ to $1\cdot7200$.

The most important atomic refractions for the D-line, which are needed for statements on the constitution of organic compounds, are as follows :—[2]

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[1] W. A. Roth and F. Eisenlohr : " Refraktometrisches Hilfsbuch," Leipzig, 1911.

[2] Landolt-Börnstein : 5th edition, Berlin, p. 985, 1923. Fuller data for further atoms and groups of atoms as well as the numbers for other wave-lengths (dispersion) are given.

C	.	.	. 2·418	O (hydroxyl)	.	. 1·525
H	.	.	. 1·100	O(ether)	.	. 1·643
Cl	.	.	. 5·967	O (carbonyl).	.	. 2·211
Br	.	.	. 8·865	N (prim. amino)	.	. 2·322
I	.	.	. 13·900	N (sec. amino)	.	. 2·502
Double bond	.	. 1·733	N (tert. amino)	.	. 2·840
Triple bond	.	. 2·398	N (nitrile)	.	. 3·118

Example :—

Benzene. $M = 78·05$; $n_D^{20} = 1·5009$; $d_4^{20} = 0·879$.
MR, calculated $= 6C + 6H + 3F = 26·31$.
MR, found $= 26·16$.

VI. Determination of the Absorption Spectrum by the Method of R. W. Pohl

Photoelectric absorption photometry is based on the photo-electric cell of J. Elster and H. Geitel.[1]

Inside a high-vacuum glass bulb *Z* (Fig. 69), there is a surface of an alkali metal, which is negatively charged through a battery *E*. If a beam of light now falls on this surface, electrons are there liberated, which are conducted to a second electrode by means of an induced E.M.F., due to the action of the electric field; from there, they pass into the extremities of a sensitive fibre-electrometer A_1. The case of the electrometer is connected with the positive pole of the battery *E*. The photoelectric current which results from the exposure can therefore be measured by the charging of the electro-meter during a definite time. The progress of the charging is shown by the throw of the electrometer fibre along a scale. The number of the electrons split off, and therefore the strength of the photo-electric current, expressed by the charging of the electrometer during a definite time, is directly proportional to the intensity of the light in the photo-cell.

If one places the solution to be examined in front of the photo-cell, in the path of the light, the monochromatic waves of the measuring light are absorbed during their passage through the solution, by the molecules in it, in a way characteristic of the solu-tion. The measurement of intensity of the light passing through is always carried out against the solvent then used, so that losses due to reflection and absorption in the solvent are eliminated. The changes in intensity due to the dissolved molecules are then directly proportional to the throw of the calibrated electrometer.

From the comparison of the intensities of the light passing

through the solution or through the solvent one can calculate the so-called molar absorption constant. This is obtained from the Lambert-Beer Law, which is the basis of the whole of absorption photometry. This law states that the decrease in intensity of the light entering every layer-element is proportional to the concentration of the absorbing material and to the intensity of the light. The absorbing molecules should thus not vary with alterations in concentration.

This law is expressed by the following formula :—

$$I = I_0 \cdot e^{-\varkappa \cdot d}.$$

I_0 is the intensity of the light falling on the layer d, I the intensity of the light passing through it, $\varkappa$ the molar absorption constant, *i.e.*, the absorption constant for 1 gm.-molecule per litre of the substance concerned. The absorption constant $\varkappa$ also corresponds to the reciprocal value of that layer-thickness, which with a concentration $c = 1$ weakens the intensity of the incident light of a definite wavelength to one-tenth of the original. The equation may be written :—

$$\varkappa = \frac{1}{c \cdot d} \ln \frac{I_0}{I} = \frac{2 \cdot 3}{c \cdot d} \log \frac{I_0}{I} \tag{I}$$

The scale divisions of the electrometer throw, read off with alternate insertion of the pure solvent and the solution, give the intensities I_0 and I directly. The thickness of the layer d is known from the dimensions of the absorption vessel. The concentration c is calculated from the weight.

By this method one can calculate the molar absorption constant for every wave-length of the light which passes through and the values may be recorded on squared paper. The abscissæ give the wave-lengths in mμ, the ordinates the molar absorption constants $\varkappa$ in cm.$^{-1}$ (Fig. 70). By joining the separate points a curve is obtained which gives information on the absorption behaviour of the substance concerned. The measurements are extended to the greatest possible range of wave-lengths.

The Photoelectric Spectrum Photometer of R. W. Pohl

For the measurement of the absorption of light with the help of photoelectric cells [1] different methods are used.[2] The photoelectric spectrum photometer of R. W. Pohl,[3] which will here be described, has been found extremely useful for years in the I. Physical Institute

[1] A full treatment of the technique of absorption spectrophotometry, particularly of that on a photographic basis, and a review of the range of use of the different methods, is given by E. Twyman and C. B. Allsopp : " The Practice of Absorption Spectrophotometry," London (A. Hilger, 1934).

[2] H. V. Halban and H. Geigel : *Z. physik. Chem.*, **96**, 214 (1920). R. Pohl : *Naturwiss*, **15**, 433 (1927). H. Rosenberg : *Naturwiss*, **9**, 359 (1921).

[3] R. W. Pohl : *Gött. Nachr. Math.-physik. Kl.*, 1926, 185.

of Göttingen University and in the Physical Section of the Kaiser-Wilhelm-Institut for medical research in Heidelberg.

The apparatus may be obtained from Spindler and Hoyer, Göttingen. From the complete prospectus of this firm fuller details may be obtained, as, *e.g.*, on the erection of the apparatus, the optical adjustment, the connections, etc.

R. W. Pohl's spectrum photometer consists of a double mono-chromator[1] and a photoelectric photometer; two models are required, for measurements in ultra-violet light and in the visible

FIG. 69.—R. W. Pohl's Photoelectric spectrum photometer.

F, Light-source. P_1, P_2, Prisms. S_1, S_2, Diaphragms. A_1, A_2, Filament electrometers. Z_1, Z_2, Photo-cells.
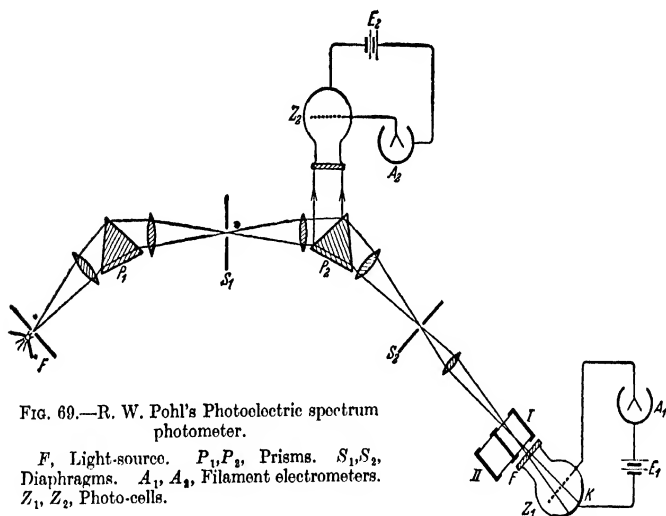
spectrum. The arrangement for the ultra-violet is evident from Fig. 69 :—

The rock salt prism P_1 and the quartz prism P_2 are each on a prism-table with automatic regulation of minimum deviation. Both prism-tables are connected together by a three-sided iron support and each carries, besides, a second support which can be displaced horizontally (not shown in the illustration). On these supports the further individual parts with riders are set up (see the accurate illustration in the firm's prospectus). As the source of light F, a mercury-quartz lamp serves for the long-waved ultra-violet, and spark spectra of cadmium, gold, aluminium, and magnesium for the short-waved ultra-violet. The source of light is in a box and the light passes through a slit. Because light which is spectrally very clean is required, on account of the fluctuation of the sensitivity of the photoelectric cell, one does away with all adjoining light by

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[1] H. Lehmann : *Ann. d. Phys.* (4), **5**, 633 (1901). G. Rudert : *Ann. d. Phys.* (4), **31**, 559 (1910).

double spectrum analysis and by sharp stopping. Both prisms ("double-monochromator") serve for this, as do also the diaphragms S_1 and S_2. With the help of a lens, the image of the slit S_2 is focussed on the window of the cell. The measuring light thus passes through the bulbs I (solution) and II (solvent) into Z_1, which is in connection with the electrometer A.

According to the intensity of the light given up through the bulbs, a slower or faster throw of the electrometer fibre takes place. For the cutting out of the fluctuations in intensity produced through variability of the source of light during the measurement, the small part of the measuring light reflected at the surface of the prism P_2 is led to a second photocell Z_2, which in its turn is again connected with an electrometer A_2. The throw of this electrometer is then compared with that of the first electrometer A_1 and the fluctuations in intensity of the light are thus taken into account. Both electrometers are calibrated and a throw of about one scale division corresponds to 1/10–1/20 volts between fibre and case. If the rise of the divergence with the voltage is not linear, a calibration curve is to be plotted.

The apparatus for measuring absorption in the visible spectrum differs from that described above in that the three-sided bars are all fixed (thus they cannot be displaced), and in the lack of the second photoelectric photometer. The prisms here consist of flint glass. The light of a Nernst-burner is used as the source of light; owing to its steadiness the second photometer is superfluous. The individual lines of the spectrum at intervals of 5 mμ are obtained by displacement of the slit S_1, which rests on a precision rider with a calibrated micrometer scale. The beat of a metronome is used for timing the throw of the electrometer fibre.

Carrying out the Measurement

In distinction from other methods of optical measurement, *e.g.*, the determination of polarisation and refraction, which can easily be carried out with little expenditure on apparatus and modest knowledge of physics, the measurement of the absorption spectrum belongs to the sphere of the physicist. If, however, the apparatus has once been set up and already tested frequently, the chemist is also given the opportunity of learning and mastering this method. This, however, requires much practice and a certain aptitude. The exceedingly sensitive electrometer must be treated with special care. For this, attention must be concentrated especially on the very various intensities of some spectrum lines; the intensity is to be regulated by altering the breadth of the slit S_1 or S_2.

Usually 1·5–2 mgm. of absolutely pure substance are weighed,

on the micro-balance, into a 25 c.c. measuring flask from a weighing tube with a handle. After orientating above the solubility ratio of the substance, the solvent is added up to the mark and mixed well, after which it must be seen that all is dissolved. If only a very small amount of material is available, the same concentration can naturally be obtained, *e.g.*, by dissolving 0·5 mgm. in 10 c.c. The weight is adjusted also according to the absorption strength of the substance concerned. This can be ascertained when one places the bulb with the solution immediately in front of a small plate of uranium glass which is held just in front of the slit S_1 in the path of the rays, and sees whether the lines concerned still pass through.

If a band is concerned, which at the given concentration only passes through very weakly or not at all, a higher concentration is used. In order that, for example, the absorption band of the longest wave-length (413 mμ), of azobenzene may be characterised, one must dissolve about 12 mgm. of the substance in 20 c.c. of alcohol.

If the concentration which has been selected is too high, so that all light is absorbed in the course of the measurement, one only needs to dilute with the solvent and to re-determine any measured point which was obtained with the original concentration. One is thus in a position, by keeping the preceding measurements, to convert all results to the new concentration.

For measurement in ultra-violet light, the solution which is kept in the measuring flask is poured into a quartz bulb I of known thickness (in our case, 0·114 cm.). To avoid oxidation of sensitive substances by the air in ultra-violet light, the bulb has a double seal.[1] In a second quartz bulb II of the same dimensions the pure solvent is placed. Both absorption vessels are placed immediately in front of the photocell Z_1, on a rider with movable pillar. This arrangement permits I and II to be alternately inserted in the beam of light. To avoid reflection, the bulb must be exactly perpendicular to the beam of light.

The room is now darkened, the mercury lamp or sparking source of light is operated, and one assures oneself of the sharpness of the separate lines of the spectrum by means of a small plate of uranium glass (see prospectus) which is held in front of the slit S_1, obliquely in the beam of light. To obtain this sharpness, the lens between P_1 and S_1 may eventually be slightly displaced. To discover the individual lines quickly, the adjustment must have been made only above the mercury spectrum or the individual spark spectra. If one is above the position of particular lines in the image, one can easily focus the required lines sharply in the centre of the slit S_1 through horizontal displacement of the box with the source of light,

[1] A. Smakula : *Gött. Nachr. Math.-physik. Kl.,* 1928, p. 1.

on the arm which can be moved. One usually begins with the mercury line at 365 mμ. One must also assure oneself the light falls fully on the lens and not obliquely. The further accurate and sharp adjustment of the path of the light follows from displacement of the other movable arm, on which is the photo-cell, and through small adjustment of the lens. A sharp image of the lines of the spectrum is thus obtained in the plane of the slit S_2. One now moves the bulb containing the solvent (*II*) into the beam of light. The slits S_1 and S_2 are set according to the intensity of the line used and are previously regulated, from control tests with the electrometers. With the great sensitivity of quartz filaments the

TABLE I. THE WAVE-LENGTHS OF THE MOST IMPORTANT
ULTRA-VIOLET LINES IN Mμ

Wave-length mμ.	Metal.	Wave-length mμ.	Metal.	Wave-length mμ.	Metal.	Wave-length mμ.	Metal.
365	Hg	296	Hg	248	Hg	214	Cd
360	Cd	289	Hg	240	Hg	211	Au
346	Cd	280	Hg	238	Hg	208	Au
340	Cd	275	Hg	234	Hg	204	Au
334	Hg	270	Hg	232	Cd	200	Au
326	Cd	265	Hg	230	Hg	199	Al
313	Hg	257	Hg	226	Cd	193	Al
309	Mg	254	Cd	219	Cd	186	Al
302	Hg						

greatest care is necessary in order to prevent them from jamming tightly.

The actual measurement can now begin; the photographic shutter behind the slit S_1 is closed. This shutter is released by a wire. With the spark spectra the shutter always remains open. Both electrometers are now earthed. If the threads remain in contact, then no disturbances are present. One now opens the shutter or inserts the spark spectra. Immediately the electrometer A_2 has travelled a definite distance, *e.g.*, 20 scale divisions, one breaks the exposure and reads the throw of the other electrometer A_1. The scale divisions of A_1 give I_0 directly. The bulb with the solution (*I*) is now inserted and a similar determination is made, thus I is found. If the throw increases in proportion to the voltage, the ratio I_0/I, may be found directly; otherwise, the voltages which correspond must be deduced from the calibration curve.

By this method one extends the measurements always further towards the shorter wave-lengths, through fresh adjustment of the next lines of the spectrum, till the solvent used no longer permits

the passage of light, *i.e.*, specific absorption by the solvent enters. If possible, the solvents used are such as only absorb very short waves, *e.g.*, hexane, alcohol, or water.

The visible spectrum range is used if an absorption of the solution in the visible spectrum is anticipated on the ground of the colour or, better, from the course of the measurement of absorption in ultra-violet light. For this, the absorption vessel is placed in front of the photo-cell Z_1 as before, the Nernst-pencil, which serves as source of light, is lit and the slit S_1 is regulated with the help of the measuring-drum, according to the calibration table, for the passage of light of definite wave-length, beginning at 370 mμ. The bulb with the solvent is now pushed in front of the photo-electric cell, the pendulum of the metronome is set going, the photographic shutter is opened, and the throw of the electrometer fibre during an interval of time indicated by the metronome is observed. Thus, after four swings of the pendulum the exposure may be broken and the scale divisions I_0 read. Similarly I is obtained. The slit S_1 is adjusted by the micrometer drum for the next wave-length, which is 5 mμ higher, and I and I_0 are read similarly for this. This is continued, increasing the wave-length by 5 mμ each time, till the solution no longer absorbs the wave-length which is passed through. From the ratio I_0/I the molar absorption constant for the individual wave-lengths is then calculated, as can best be seen from an example.

Example and Calculation

Mr. A. Schröder carried out determinations of the molar absorption constant of lactoflavine (vitamin B_2) for the different regions of the ultra-violet and the visible spectra and drew the absorption curve. This was done in the Physics Section of the Institute of this place.

Extremely pure lactoflavine[1] was weighed, on the micro-balance, into a measuring flask of 10 c.c. capacity; this was filled to the mark with water, in which the substance dissolved completely on mixing well. The weight taken was 0·855 mgm. and the molecular weight is 376. The concentration $c = \dfrac{0.855 \times 100}{1000 \times 376} = 2.27 \times 10^{-4}$ gm.-molecules per litre. The thickness of the layer $d = 0.144$ c.c. The factor $f = \dfrac{1}{c} = \dfrac{1}{2.27 \times 10^{-4}} = 4.4 \times 10^3$. According to equation (I), $\varkappa = \left(\dfrac{4.4 \times 2.3}{0.114}\right)\left(\log \dfrac{I_0}{I}\right)10^3$. With the first term of

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[1] The preparation of m.p. 293° was originally made by H. Rudy; *cf.* R. Kuhn, H. Rudy and R. Weygand : *Ber. dtsch. chem. Ges.*, **68**, 625 (1935).

the equation, which holds for all the measurements, one uses only the logarithms of the intensity ratio of the different wave-lengths for the multiplication.

TABLE 2

Wave-length mμ.	I_0 (Scale-divisions).	I (Scale-divisions).	I_0 (Scale-divisions).	$\log \frac{I_0}{I}$.	$\varkappa/10^3$.
510	33·1	32·4	33·1	0·009	0·8
500	32·9	30·0	32·9	0·040	3·44
490	32·0	26·0	32·0	0·090	7·7
480	32·3	21·1	32·3	0·185	16·0
475	33·9	20·1	33·9	0·227	19·5
470	30·9	17·0	30·9	0·260	22·4
465	29·1	15·1	29·1	0·285	24·4
460	33·0	16·9	33·0	0·290	25·0
455	33·1	15·8	33·1	0·322	27·6
450	30·1	14·1	30·0	0·330	28·4
445	32·9	15·1	32·9	0·338	30·0
440	28·1	13·0	28·1	0·334	29·6
435	30·1	14·0	30·0	0·331	29·4
430	21·0	10·5	21·0	0·301	26·7
425	32·0	17·0	32·0	0·274	23·5
420	33·0	18·0	33·0	0·263	22·6
415	30·9	17·8	31·0	0·239	20·5
410	30·0	18·1	30·0	0·220	18·9
400	30·7	20·0	30·8	0·188	16·2
390	34·9	21·2	34·9	0·216	18·5
380	18·1	10·0	18·1	0·257	22·0
375	9·0	5·0	9·0	0·255	21·9
370	4·0	2·3	4·0	0·243	20·9
365	33·0	24·0	33·0	0·158	18·75
360	30·7	19·6	31·0	0·216	18·6
346	31·3	20·4	31·3	0·190	16·4
340	30·8	21·6	30·9	0·168	14·5
334	30·3	22·1	30·3	0·147	12·7
326	29·9	23·8	29·9	0·109	9·4
313	30·0	27·2	30·0	0·050	4·32
302	30·0	28·0	30·0	0·035	3·0
289	30·7	20·4	30·6	0·188	16·2
280	31·8	11·0	31·8	0·467	40·0
275	30·3	6·9	30·3	0·654	56·4
270	32·0	5·0	32·0	0·814	69·9
265	29·0	4·0	29·0	0·844	72·5
254	31·1	7·3	31·0	0·635	54·8
248	31·0	12·3	31·0	0·408	35·0

In the above table, *e.g.*, for $\lambda = 510$ mμ, $\varkappa = 0·8 \times 10^3$. The corresponding values for λ and $\varkappa$ are shown on a graph (Fig. 70).

The lactoflavine absorption curve shows three marked maxima,

which lie at 266, 378, and 445 mμ and at a height of 73, 22, and 30 times 10^3 cm.$^{-1}$. The absorption band at 445 mμ, which lies in the visible spectrum, causes the yellow colour of the substance. Its height is a criterion of the purity. One is in a position, through measurement of the absorption of the single point at 445 mμ, to check the purity of the substance, *i.e.*, to determine the percentage of impurities present, if these do not accidentally occur in the same region of absorption. This orientating and indeed clear and quantitative measurement is to be arranged with very small expenditure in substance and time, in contrast with an elementary analysis or a biological test method. The melting-point has not the
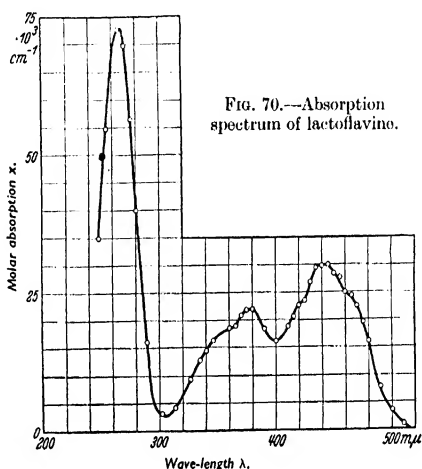
Fig. 70.—Absorption spectrum of lactoflavine.

great sensitivity which is necessary for checking the complete purity of the natural product.

General ; Determination of Constitution

The importance of photoelectric absorption photometry lies especially in its great accuracy and sensitivity. With the help of R. W. Pohl's apparatus, well-marked maxima can be determined to $\lambda \pm 1$ mμ. The limiting error for the height of the band lies around ± 2 per cent. Even the smallest amounts of impurity, which can scarcely be detected by other physical properties—*e.g.*, melting-point—may exert a clearly visible influence on the height of the bands.

With the interpretation of the absorption spectra it must always be kept in mind that all expressions are based in great measure on conclusions from analogy which are drawn from the spectra of a large number of materials for comparison. They thus possess a

purely empirical character. Meantime it is not possible to deduce the absorption behaviour of chemical compounds from their structural formulæ, as molecular refraction, for example, may be deduced. This can only be obtained ultimately through increase in the number and accurate sifting of the materials observed.[1] Among other work, that of K. W. Hausser and his colleagues [2] on light absorption and double linkage of homologous series, forms a very promising beginning. If, in many cases, difficulties occur even through the influence of the solvent, the action of influences which have not yet been investigated cannot be excluded from consideration. If definite absorption maxima and intensities are indeed due to the different chromophores, these may yet influence one another reciprocally. Also many cases occur, where, in spite of constitutional difference of two compounds, their absorption spectra show scarcely any difference. For this reason, Ley [3] is entitled to remind us not to omit the connecting of this method with other physicochemical and purely chemical methods for the determination of the constitution.

Calculation of the Results of Microanalyses

This is always carried out logarithmetically, using exact atomic weights and F. W. Kuster's logarithmic tables for chemists. It is particularly emphasised in the " explanations," which Thiel gives to Table 5 in the editions 35–40 of his booklet (pp. 120–125), that one should never avoid the trouble of calculating the percentage of all the solvents from the formula, even although one or two elements only are in question. Thus the percentage of the oxygen should always be calculated. The accuracy of the calculations can then be checked, by ascertaining that the sum of the percentages amounts to 100. The necessary factors for the calculation of the analyses, and their logarithmic mantissæ, will now be given in the form recommended by Kuster.

As factors for the determination of methoxyl, ethoxyl, methyl and carboxyl groups are missing in Kuster's logarithmic tables, these are also given here, as they are frequently used in calculating formulæ.

[1] In connection with this, see H. Ley's contribution, " Relation between absorption and chemical constitution," in H. Geiger and K. Scheel : " Handbuch der Physik," vol. 21, pp. 57–165 (Berlin, 1929).
[2] Partially summarised by K. W. Hausser : *Z. techn. Physik.*, **15**, 10 (1934) ; K. W. Hausser, R. Kuhn, and colleagues : *Z. physik. Chem.*, Section B, **29**, 363–389 (1935).
[3] See K. v. Auwers : *Ann.* **505**, 292 (1933).
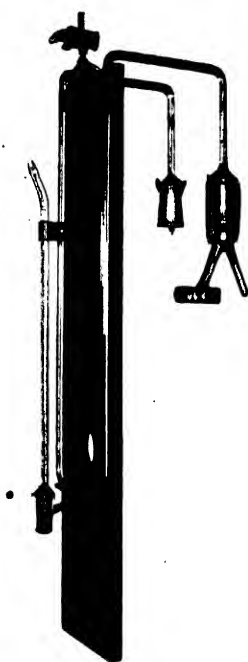
FIG. 71. --Hydrogenation vessel, with manometer connection fixed to the
shaking board.
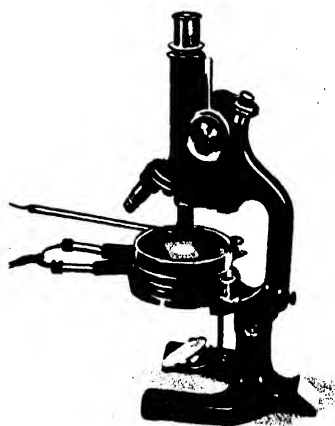
FIG. 72.—Melting-point apparatus with heating tube, of L. Kofler and H. Hilbck.
[*Arch. Pharmaz.* **270**, (1932)]

Gravimetric Factors

Required.	Found.	Factor.	Log Factor.
C	CO_2	0·2727	43573
H	H_2O	0·1119	04875
Cl	AgCl	0·2474	39334
Br	AgBr	0·4255	62894
I	AgI	0·5406	73284
S	$BaSO_4$	0·1373	13782
P	$MgNH_4PO_4 . 6$ aq	0·1264	10164
P	$Mg_2P_2O_7$	0·2786	44499
P	Amm. phos. molybdate	0·014524	16209
P_2O_5	Amm. phos. molybdate	0·03326	52192
As	$Mg_2As_2O_7$	0·4827	68371
$O.CH_3$	AgI	0·1321	12096
$O . C_2H_5$	AgI	0·1918	28287
CH_3	AgI	0·06398	80604
C_2H_5	AgI	0·12380	09273

Table on the Forms of Determination of Metals in Organic Salts

Required.	Found.	Factor.	Log Factor.
Silver . . .	Ag	—	—
Aluminium . .	Al_2O_3	0·5291	72357
Gold . . .	Au	—	—
Barium . . .	$BaSO_4$	0·5885	76972
Bismuth . .	Bi_2O_3	0·8970	95279
Calcium. . .	$CaSO_4$	0·2944	46889
Cadmium . .	$CdSO_4$	0·5392	73176
Cobalt . . .	Co	—	—
Chromium . .	Cr_2O_3	0·6843	83522
Copper . . .	CuO	0·7989	90250
Iron . . .	Fe_2O_3	0·6994	84473
Potassium . .	K_2SO_4	0·4488	65203
Lithium . .	Li_2SO_4	0·1263	10123
Magnesium . .	MgO	0·6032	78044
	$MgSO_4$	0·2020	30541
Manganese . .	$MnSO_4$	0·3638	56086
Sodium . . .	Na_2SO_4	0·3238	51026
Nickel . . .	Ni	—	—
Lead . . .	$PbSO_4$	0·6833	83458
Platinum . .	Pt	—	—
Silicon . . .	SiO_2	0·4672	66950
Tin . . .	SnO_2	0·7877	89634

Volumetric Factors

Required.	c.c. of Standard Solution used.	Factor.	Log Factor.
As	$N/100-Na_2S_2O_3$	0·3748	57330
Br	$N/100-NaOH$	0·7992	90266
Cl	$N/100-NaOH$	0·3546	54974
I	$N/50-Na_2S_2O_3$	0·4231	62644
N	$N/100-HCl$	0·1401	14638
S	$N/100-NaOH$	0·1603	20493
	$N/50-NaOH$	0·3206	50596
CH_3	$N/50-Na_2S_2O_3$	0·050078	69964
C_2H_5	$N/50-Na_2S_2O_3$	0·096796	98586
$CH_3(C)$	$N/100-NaOH$	0·15023	17676
$C_3H_6 =$	$N/20-Iodine$	0·3505	54469
$- O . CH_3$	$N/50-Na_2S_2O_3$	0·10341	01458
$- O . C_2H_5$	$N/50-Na_2S_2O_3$	0·15013	17647
COOH	$N/100-NaOH$	0·45008	65323
$CH_3 . CO-$	$N/100-NaOH$	0·4302	63370
$C_5H_6 . CO-$	$N/100-NaOH$	1·0504	02135
$CH_3 . COOH$	$N/100-NaOH$	0·60031	77838

Multiples of

$O.CH_3$	Log	$O.C_2H_5$	Log
1. 31·02 (34)	49169	1. 45·04	65360
2. 62·05	79273	2. 90·08	95463
3. 93·07	96881	3. 135·12	13072
4. 124·10	09376	4. 180·16	25568
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Literature

Author.	Title.
ABDERHALDEN, E. .	" Handbuch der biologischen Arbeitsmethoden." Berlin : Urban & Schwarzenberg, 1919.
BOËTIUS, M. . .	" Über die Fehlerquellen bei der mikroanalytischen Bestimmung des Kohlen- und Wasserstoffes nach der Methode von Fritz Pregl." Berlin : Verlag Chemie G. .m. b. H., 1931.
EMICH, F. . .	" Lehrbuch der Mikrochemie." Munich : J. F. Bergmann, 1926.
EMICH, F. . .	" Mikrochemisches Praktikum." Munich : J. F. Bergmann, 1931.
FRIEDRICH, A. .	" Die Praxis der quantitativen organischen Mikroanalyse." Leipzig and Vienna : Franz Deuticke, 1933.
KLEIN, G. . .	" Handbuch der Pflanzenanalyse." Berlin : Julius Springer, 1931.
KOLTHOFF, I. M. .	" Die Massanalyse." Berlin : Julius Springer, 1928.
KÜSTER, F. W. .	" Logarithmische Rechentafeln für Chemiker." Berlin and Leipzig : Walter de Gruyter & Co.
LANDOLT-BÖRNSTEIN	" Physikalisch-chemische Tabellen, 5 Aufl." Berlin : Julius Springer.
LINDNER [1], J. .	" Mikro-massanalytische Bestimmung des Kohlenstoffes und Wasserstoffes mit grundlegender Behandlung der Fehlerquellen in der Elementaranalyse." Berlin : Verlag Chemie G.m. b. H., 1935.
RONA, P. . .	" Praktikum der physiologischen Chemie. Berlin : Julius Springer, 1929–1931.
ROTH, W. A. und F. EISENLOHR	" Refraktometrisches Hilfsbuch." Berlin and Leipzig : Walter de Gruyter & Co., 1911.
WEYGAND, C. . .	" Quantitative analytische Mikromethoden der organischen Chemie. Leipzig : Akademische Verlags-Gesellschaft m. b. H., 1931.

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